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The politics of biomedical research

For the past eighteen months, the Nixon Administration and the biomedical research community in the United States have been engaged in a verbal battle over the management and funding of medical research. Some fundamental principles are at stake, and some deep divisions—which have resulted in the dismissal or resignation of senior officials of the National Institutes of Health—have occurred. It is important, for the health of the biomedical research effort in general, and for the future of NIH in particular, that the differences of opinion should be resolved. But how?

The nub of the dispute is this. For years, the National Institutes of Health has enjoyed considerable autonomy. It has largely been sheltered from policy decisions imposed from the White House, or from the headquarters of its parent department, the Department of Health, Education and Welfare. It has established a well-deserved international reputation, and its efforts have contributed to advances in the treatment of numerous diseases. But its budget has grown to the point at which the Office of Management and Budget is understandably asking what return the Administration is getting on its investment in biomedical research. Policy decisions vitally affecting the day-to-day operations of NIH are increasingly being made in OMB and HEW, and NIH officials and scientists therefore complain of a loss of autonomy.

Superimposed on this drift toward increased budgetary control from outside NIH is a move toward what NIH scientists refer to as 'politicalisation of research'. In the past two years, Congress has passed two bills designed to increase funding for research on cancer and diseases of the heart and lungs. The result has been that the National Cancer Institute and the National Heart and Lung Institute have both enjoyed large funding increases, while other institutes have either had their budgets cut or held constant. Moreover, an increasing proportion of the funds channelled through NIH are spent on contracts rather than the traditional grants. Contracts provide a mechanism by which research projects can be more tightly controlled. NIH scientists argue that these trends have unbalanced the overall biomedical research programme, and have taken money away from the individual university scientist—the man who has traditionally provided most of the main breakthroughs in research.

In a recent letter to the *Washington Post*, Dr Charles C. Edwards, Assistant Secretary for Health in the Department of Health, Education and Welfare, argued that NIH can no longer expect to have the autonomy it once enjoyed. Research money should be targeted on problems whose solution would have the greatest benefit for society, he argued, and the Administration has every right to expect a return on its investment.

The scientists replied that while the amount of money to be devoted to biomedical research is indeed a political question, the manner in which it is spent should be decided entirely on scientific grounds.

Both viewpoints are eminently reasonable, but can they be reconciled? Last week, Dr Edwards spoke to scientists at NIH, and according to reports, he reassured them that scientists, not politicians, would always have the final say on how research funds should be spent. Although much of his talk offered such reassurances, scientists who attended came away unconvinced that their case has really been accepted by the Administration. And their fears were fueled by the fact that the Administration's budget for 1975 carries through the trend of increasing expenditures on cancer research and heart and lung research, while holding other areas constant. Such distrust indicates the need for new planning mechanisms to be introduced for biomedical research.

Last week, Senator Edward Kennedy offered at least a step in this direction (see page 4). He has suggested that a three-member council should be set up to report directly to President Nixon on the overall balance and content of the biomedical research programme. Such a body would at least provide some insulation between NIH and the rest of the Administration. At all costs, however, the danger that it would simply turn into an outlet for the scientists' complaints and thus generate more heat than light, must be avoided. Whether that will happen will depend to a large extent on who is appointed.

100 years ago



THE young King of Siam having come of age on October 10 last, great feasts were given to his subjects at Bangkok, the chief town of his dominion. Amongst other attractions was the ascent of a small mounted balloon, which had been constructed in Paris and had arrived by steam a few days previously. Liberal offers were made to procure an *aéronaut*, but were of no avail, nobody amongst the Siamese presuming to ascend. Consequently his Majesty ordered a slave, selected from amongst the less heavy of his household, to be sent up in the car. In order to encourage the poor *aéronaut*, so frightened for his life, he was promised to be rewarded with his enfranchisement. The ascent took place and elicited much enthusiasm from the bystanders; but, unhappily, nothing was heard from the poor fellow or of the craft.

From *Nature*, 9, 350, March 5, 1874.



international news

Protest about emigration from the Soviet Union

Vera Rich, London

THE right of the Jewish intelligentsia to emigrate from the Soviet Union to Israel has been raised once again by the fact, in Moscow, of David Azbel, a professor of chemistry, Vitalii Rubin, an eminent Sinologist, and Vladimir Galatskii, a talented but little known artist. In a statement issued on the first day of their protest, the three participants declared:

"We begin today a hunger strike, taking a step befitting political prisoners. We consider ourselves as such even though we live in Moscow in our flats and with our families. One can deprive a human being of his liberty even without arresting him. The case then, is of arrest without investigation, without a trial and without a date for release".

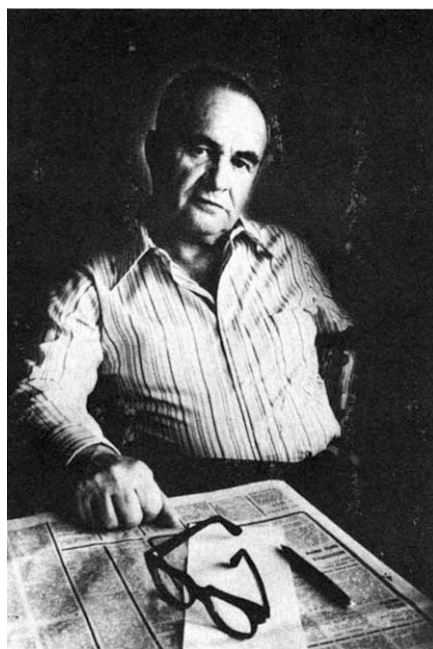
Since October 1971 when the Soviet restrictions of Jewish emigration to Israel were relaxed insofar as total numbers were concerned, it has become increasingly clear that the new policy was not to be applied on a basis of equality. According to a law of 1942, all persons wishing to emigrate from the Soviet Union are obliged to pay 400 roubles for an exit visa, plus 500 roubles for renouncing their Soviet citizenship—900 roubles in all (£450). In addition to this, however, a new principle was introduced on August 3, 1972—that all persons with higher education wishing to emigrate should first refund to the state the cost of that education.

In a press statement made through the Novosti agency (December 28, 1972) Deputy Minister of the Interior Boris T. Shumilin explained this policy in terms of economic pragmatism with a touch of socialist solidarity. "The Soviet Union", he said, "has no intention of acting as a philanthropist towards persons on whose education the state has spent considerable sums, or towards those capitalist states to which these persons are emigrating".

For this reason the repayment of fees is not demanded from those departing for socialist countries or those which have "taken the road to independent development", since special agreements exist between the Soviet Union and

these countries concerning the repayment of fees. Persons of retirement age and invalids are, he said, exempt from all repayments, and rebates are given in accordance with the length of time worked (for example, 75% rebate for a man with a working record of 25 years or a woman with a working record of 20 years).

One would expect that since the state demands such high 'transfer fees' for its valuable personnel (a record figure of 23,000 roubles paid by an unnamed Jewish composer was reported in February, 1973), it would use these human 'assets' in the best interests of the Soviet economy. This, however, is not the case. Although it is not illegal to apply for



David Azbel

emigration to Israel, clearly those who do so are dissatisfied with the Soviet regime. And dissatisfaction with the regime is viewed by the authorities as dissent and, in many cases, as mental illness.

Thus one has the anomaly that a scholar such as philologist Vitalii Shevoroshkin, refused an exit visa on the grounds that his services were "indispensable" to the Soviet Academy of Sciences, was shortly afterwards dismissed from his post. One presumes that no adequate replacement can be found!

Since March 1973, when the enforcement of this 'diploma tax' threatened to jeopardise Soviet-United States trade

agreements, it would seem that the repayment of higher education fees has no longer been enforced. But the journalist Victor Louis, who seems to have a special insight into the workings of the KGB, informed the Israeli newspaper *Yediot Aharonot*, that "although it had not been abolished, the tax would not in future be demanded". Inverting the proposition, this means that although for those intellectuals fortunate enough to be allowed to leave as a sop to opinion abroad (five left with much publicity at the time of the said trade talks), it remains unrevoked and could be reimposed at any time.

Instead of the diploma tax, recent policy has been one of ever increasing harassment of the would-be emigrant. He is seen as a dissenter and dismissal from his post is the least that he can expect. In the case of the electrochemist Dr Venyamin Levich, his own dismissal was followed by the exiling of his astrophysicist son to Siberia (in spite of suspected cancer of the stomach) and a curious attempt to impose the "academic oblivion" of Stalinist days.

The distribution of foreign scientific journals has been handled by the Institute of Information, which photocopies the journals and then distributes these copies to libraries and subscribers. This means that 'undesirable' material can be removed at this stage and, in order to cover up the omission, replaced by innocuous material such as book reviews from other issues.

In reproducing the *Journal of the Chemical Society*, No. 8, 1973, (which makes reference to Levich's work) the older blanking-out type of censorship was, however, adopted, so that references to the "Levich-Dogonadze" theory become references to the "... Dogonadze" theory throughout the Soviet version, except in one case where Levich's name remains by an apparent oversight (see Fig. 2).

Professor David Azbel, one of the three participants of the present 'hunger strike' was 61 years old when he applied, in May 1972, to emigrate to Israel and therefore, according to the Shumilin statement, should have qualified for emigration without payment of the diploma tax. His life history up to this time reads like a synopsis of some Solzhenitsyn novel. Orphaned at the age of nine—he was forced to watch his mother hanged during a White pogrom in the Civil War—he made his way as

A number of theories of oxidation-reduction and electrochemical electron exchange reactions have appeared. Common to several of these theories is a particular approximation method embodied in the use of the dielectric continuum representation of the electrically polarizable nature of the solvent system surrounding the reactants and products: the electron donor and acceptor species. In addition, one theory is fundamentally quantum mechanical; this theory has been developed to a considerable extent by Dogonadze and their associates.¹⁻⁴ The Dogonadze solution electron transfer theory¹ resembles the solid state polaron theory.⁵ Further, the Dogonadze theory owes much to the Born-Oppenheimer adiabatic separation of the transfer electron dynamics from environmental motions for its progress from the conceptual model, with its associated Hamiltonian operator, to the expression for the rate constant. The advantage in the application of the Born-

one of the numberless 'waifs of the Revolution' from his native Ukraine to Moscow, where distant relatives gave him a home. He graduated in 1935, just in time to be arrested in the first wave of Stalin purges. He was released in 1951 and was 'exiled' back to Ukraine, where he worked as an engineer until he was dismissed from his post in the wave of anti-Semitism at the time of the alleged "Doctors' Plot" against the life of Stalin.

After Stalin's death, he was 'rehabilitated', allowed to return to Moscow, where he completed his postgraduate studies, becoming a doctor of technical sciences in 1960 and a professor in 1961. In 1968 he became head of the Institute of Chemical Spirits and Organic Products in Moscow. During this time he published some 60 scientific papers, dealing with mass transfer, bubbling regimes and the kinetics of biochemical processes.

In May 1972, at the age of 61 (and therefore exempt from any 'diploma tax' according to Shumilin's pronouncements), he applied to emigrate with his wife and child to Israel. He was immediately dismissed from his post and the long harassment started. In December 1972, he was arrested with other Soviet Jews following a hunger strike outside the Central Telegraphic Office in Moscow and was sentenced to 15 days' imprisonment for "holliganism". In October 1973 he was reported arrested in Leningrad in a general round-up of leading Jews at the time of the Yom Kippur War. A few days later, he is said to have been in hiding in his Moscow flat, under constant surveillance by the KGB and afraid to leave, even to buy food, lest he be arrested. Although these last reports seem somewhat contradictory, the general impression of harassment is unmistakable.

So, with his friends Rubin and Galatskii, he now considers himself a "political prisoner", although as yet all three live in Moscow in their own flats and with their families. Compelled in 'the interests of the state' to remain in the Soviet Union at a time when it is ready to expel such 'undesirables' as Solzhenitsyn, they are undertaking his hunger strike to draw attention to the

anomalous position of the Soviet Jewish intelligentsia. Although the official propaganda sources stress that "tens of thousands" of Russian Jews are permitted to leave, these figures, say Azbel and his companions, only mask the 'human drama' of the Jewish intelligentsia, who "From the moment we applied for exit visas . . . have been victims of endless harassment, our dignity as human beings often degraded. We have had to face officials who could torture us at will. Our application to higher officials proving our just causes were answered by lies and mockery. Our protests were answered by repression".

New riot control agent for US Army

Colin Norman, Washington

A CONTROVERSIAL new riot control agent, which was developed in Britain, has been approved for use by the United States Army. Called CR, it is a highly potent irritant to the eyes and skin, and it is likely to be at least a partial replacement for CS, the riot control agent widely used in Vietnam. The first public indication of the approval of CR for use by US forces was contained in a letter sent last week by Dr Malcolm Currie, the director of research and development in the Department of Defense, to Wayne Owens, a Democratic Congressman from Utah.

Developed at Britain's Chemical Defense Establishment at Porton Down, CR (its chemical name is dibenzoxazepine), is considered by both the British and US governments to have some advantages over CS as a riot control agent, chief of which is that it can be sprayed rather than disseminated by a smoke bomb.

CS is relatively indiscriminate in its effects—when used in a wind, it tends to blow away from the target area, and even to blow back into the faces of the troops using it. There have also been reports that rioters can develop a tolerance for its irritant effects. CR, however, can be used as a liquid suspension in propylene glycol, which means that it can be sprayed directly at the target.

The agent was issued to British troops in November last year, amid considerable criticism because the British government had released only scant information about the toxicity of the agent. No further information has yet been issued in the United States, although the most comprehensive tests were carried out at the Edgewood Arsenal in Maryland. According to Currie's letter, the US government is precluded from releasing reports of those tests because the Department of Defense "is bound by the terms of the standardization agreements not to release information derived from the property of foreign governments". He added, however, that "the UK has now embarked on an active program to present information on CR in the scientific literature. We anticipate, therefore, early release of some technical publications concerning our investigations".

The most comprehensive data so far published on CR were contained in an article in an obscure British magazine, *Medicine, Science and the Law* (October, 1973), which was written by senior officials from Porton Down. The paper described tests on 150 male volunteers, and it described the effects of CR as being very sharp and painful, and they lasted for about 20 minutes. The agent also causes the victim to shut his eyes tightly.

As for toxicity, such information as there was in the article suggests that the toxicity of the agent is low, but it was entirely inadequate to gauge the long-term effects. The key data are contained in the reports of the Edgewood Arsenal studies, and until those are released, no adequate scientific judgement of the risks associated with exposure to CR can be made. It should be noted that the Himsworth Committee, which was set up by the British government to study the use of CS, strongly recommended that all new riot control agents should be thoroughly tested and the results opened up to public debate before they are issued to the armed forces. This did not happen with CR in Britain, and the United States government has quietly followed Britain's bad example.

Should pharmaceuticals be left alone?

Roger Woodham

WHAT precisely is the Labour Party's policy for the British pharmaceutical industry? The Association of the British Pharmaceutical Industry (ABPI), for one, is not waiting any longer to find out and has published a hard-hitting riposte to that part of the Labour Party Election Manifesto that deals with future

public ownership in the industry. Predictably the association's conclusion is "leave well alone".

The manifesto devotes all too little space to spelling out just what the party has in mind. A public holding, it says, is necessary in certain industries to allow control of prices, stimulation of investment, encouragement of exports . . . There are evidently several choices.

- Nationalisation of some or all British companies manufacturing pharmaceutical products (including the British ends of multinational companies in the extreme case) .

- The setting up of a state holding company which would buy a controlling share in specified companies.

- The floating of a new company in competition with the rest.

The permutations are almost endless. Perhaps the Labour Party has in mind simply hiving off to the state certain parts of companies (declining, for example, to involve the nation in the soft drinks or cosmetics markets). In any event the 'big six'—Glaxo, Beecham, Fisons, ICI, Wellcome and Boots—are likely to feel a cold wind if the public ownership policy is ever implemented.

The ABPI argues its case against public ownership by posing and answering 13 questions. Is there any evidence, the association asks for example, that nationalisation can benefit the health of the public and the national economy? Its answer is "no", and that nationalisation would "stifle research success and reduce the industry's £220 million plus a year export record . . ." Certainly the ABPI has a point about exports, for if foreign-based companies ceased to operate in Britain about half that export potential could be lost.

This is not such an unlikely state of affairs, for any government could, if it wanted, decimate the British pharmaceutical industry in its present form by taking over one company. (Glaxo, which has a turnover of about £200 million, is a possibility for it has the added attraction of having two factories in development areas, at Alzerston in Lancashire and at Montrose in Scotland.) Given the manufacturing base the government could then invoke clauses in the 1948 Patent Act and the 1968 National Health Service and Public Health Act, which allow it to abrogate drug patents for the benefit of the public sector. Few foreign companies would stay in Britain in these circumstances. And, of course, a government buying even a part share in a foreign-based company would simply be buying bricks and mortar and a disillusional staff; no new ideas would ever again reach it from its parent.

What of research? The ABPI document boasts that the pharmaceutical industry plans to double its present

research investment during the next few years and generally expresses approval for the way in which pharmaceutical companies set about things. The objection, however, is that it is hard for a private company to justify to itself research in areas that are not seen to be potentially profitable. Presumably a national pharmaceutical corporation, or whatever, would do much less screening of compound upon compound for signs of biological activity of a desired kind, and concentrate instead on identifying problem diseases and looking, more 'scientifically', for a cure.

There is talk among Labour politicians that the British pharmaceutical research budget according to Labour plans would make the present spending look like peanuts, but there are others who would hardly bother with research at all and would concentrate on manufacturing generic drugs extremely cheaply. The national drugs bill footed by the government might be reduced from 7% of the cost of the National Health Service to 3% or 4% in this way, hardly a handsome return for such an extreme move.

The right balance may well have been struck by Pfizer, whose research effort in Britain was mainly fundamental a few years ago but is now split almost equally between the fundamental and the pragmatic.

Making out a case for the Coalplex

John Wilson

THE National Coal Board (NCB) is asking the government to provide "about £40 million" for research and development as part of a £400 million investment programme. The NCB has already carried out a great deal of fundamental research into new ways of producing energy and raw materials from coal at the Coal Research Establishment, Stoke Orchard, Cheltenham. Now it wants to set up pilot plants to assess the feasibility of its ideas on a larger scale.

Describing the diversity of the work now in progress, Dr J. Gibson, Director of the Stoke Orchard establishment, says that it ranges from the production of carbon fibre to the use of colliery spoil in motorway construction. But he outlines a unifying theme behind much of the research—the creation of a 'Coalplex'. A Coalplex is a single site where several processes for the extraction of energy from coal, usually in the form of electricity or gas, can be linked to the manufacture of other coal products such as metallurgical coke. The processes may be combined in almost any permutation so a Coalplex could provide different coal products if the emphasis on

Kennedy floats a critical plan

Colin Norman, Washington

IN an effort to provide a highly visible channel for independent criticism of the Administration's biomedical research policies, Senator Edward M. Kennedy has proposed that a three-member Presidential panel should be set up to keep an eye on the programmes of the National Institutes of Health (NIH). The proposal stems from the fact that for the past two years, the Administration has favoured the National Cancer Institute and the National Heart and Lung Institute with large budget increases, chiefly at the expense of other areas of biomedical research. Kennedy, backed by a number of influential scientists, believes that this trend is creating considerable imbalance in the overall biomedical research effort.

The panel, which was proposed in a bill Kennedy introduced last week, would be akin to the President's

Cancer Panel, a body which now oversees the national cancer programme. It would consist of three members, one of whom would be the chairman of the cancer panel, and at least two of whom would be "distinguished scientists or physicians". Its brief would be to report directly to the President and Congress on any delays or blockages in the execution of NIH's programmes—a definition sufficiently broad to allow the panel to voice its opinion on most aspects of research policy.

The bill has been sent to Kennedy's Senate Health subcommittee, where it will probably be attached to another bill which proposes some relatively minor changes in the National Cancer Act. Since it is being cosponsored by Senator Jacob Javits, the ranking Republican on the health subcommittee, there is every chance that it will be speedily approved. Meanwhile, a similar bill is being introduced into the House of Representatives by Paul G. Rogers, the chairman of the subcommittee on health of the House Commerce Committee. Again, little opposition is expected there.

the various individual processes were altered.

One part of a Coalplex could be used to supply the raw material (such as 'lean' gas of low calorific value) for several different processes and the by-products of one process could be utilised by another. In this way the overall capital cost of a Coalplex would be reduced and its efficiency increased. Dr Gibson points out that with the old town gas system, only the quantity of the products—gas, tar and coke—could be altered, not their proportion. In a Coalplex, no unwanted materials need be made.

Dr Gibson emphasises, however, that the setting up of a Coalplex represents the culmination of the research effort at Stoke Orchard rather than the core of present plans. It is the building of pilot plants to test the individual processes that is most urgent now, he says.

Perhaps one of the most important of these processes is the fluidised bed combustion of coal (FBC). The coal is burned in a sea of coal ash given the properties of a liquid by the upward passage of air. Low grade coals may be used and indeed coal washings, composed of 50% water, 25% ash and only 25% combustible material, have been successfully burned. This raises the interesting possibility of recycling some coal tips.

The equipment required by an FBC system is cheaper and more reliable than conventional apparatus, says Dr Gibson, and the process is also less harmful to the environment. Being about 10% more efficient than existing generating stations powered by coal, any FBC station would require much less water for cooling. Moreover, by adding limestone to the bed of ash, the amount of sulphur dioxide given off can be substantially reduced. With the growing concern about thermal and atmospheric pollution, Dr Gibson feels that these are important points to consider.

Because FBC is an efficient method of releasing the energy in coal, it could form a base around which a Coalplex might be built. It can also be adapted to the direct gassification of the coal, thus providing a gas from which oil can be produced. But other processes are also needed to extract the various coal products and these, too, require the development of pilot plants.

One way of obtaining coal products is to dissolve the coal in a solvent such as anthracene oil. The solution can then be used in the manufacture of carbon fibre and electrode coke. Dr Gibson claims that this coke is of sufficient quality for use in the arc processes of the steel and aluminium industries. Another extraction process which Dr Gibson would like to see in pilot operation involves the vaporisation of the coal into compressed gases held just above their criti-

cal temperature. When the resulting mixture is drawn off and cooled the coal extract can be precipitated out.

In the long term processes such as these could be the initial steps in the preparation of liquid fuels and chemicals. These might then be processed using the techniques of the petrochemical industry. With its high content of hydrogen, the gas extract is particularly suitable.

The idea of a Coalplex was first proposed by Lord Zuckerman in 1971 and most of the fundamental principles have been known for some time (aviation fuel was made from coal in Germany during the Second World War). But why has the NCB been so slow to exploit this concept? The answer lies in the economics of the operation. Quite simply it was, until very recently, several times cheaper to produce most of these materials from oil. The NCB had been thinking of an economic Coalplex in terms of 10 or 15 years from now. Recent events in the Middle East and a growing shortage of carbon products have changed this outlook. That is why the board wants to take the expensive step out of the laboratory on to the pilot site.

Nevertheless, Dr Gibson is careful to point out that he envisages a situation where coal and oil are complementary to the needs of industry and not in competition. Oil is a natural resource which contains a lot of energy and it would be a shame, he says, if such a fine material were degraded to gas, carbon fibre and other more mundane uses. If coal were used to produce these basic materials, it would relieve oil of a considerable burden.

NASA and Europe look to the planets

Colin Norman, Washington

DISCUSSIONS have been held between officials of the National Aeronautics and Space Administration and their counterparts in Europe on the possibility of a joint United States-European mission to Jupiter. During a recent visit to five European countries, NASA officials floated the idea of European cooperation with the United States on a project which would involve use of Pioneer-H, the redundant backup spacecraft for NASA's two Jupiter Pioneers. The possibility of a joint mission to the comet Eake in 1980 was also discussed. The European response was "very enthusiastic", and firm proposals are likely to be made by September.

Two chief choices are being discussed. The first would involve a launch in 1978 and Jupiter encounter in 1980 which would take the spacecraft on a

trajectory underneath the planet, to emerge north of the ecliptic plane. After Jupiter encounter, it would then go on towards the Sun, passing over the north solar pole and swinging round the Sun past the south pole. If the mission goes ahead, it would be the first spacecraft to be sent out of the ecliptic.

The second choice would be to launch Pioneer-H in 1980 on a Jupiter orbiter mission. Such a mission would extend the observations of the two Jupiter swingby missions.

Although the costs are not yet clear, the out-of-ecliptic flight would be the cheaper option. At present, Pioneer-H has a set of instruments which duplicates those carried on the Pioneer-10 and 11 swingby missions. An orbiter mission would require the development of retro rockets and some changes in instrumentation, while the Jupiter swingby, followed by the out-of-ecliptic solar flight would only require the addition of a few solar instruments.

The exact mode of cooperation is still being worked out, but as far as the orbiter is concerned, the idea would probably be that the Europeans would provide the retro rockets.

The cost of immunisation

Sally Owen

ABOUT 2,000 children suffering from vaccine damage in Britain are not eligible for compensation because of the terms of reference of the Royal Commission on Compensation and Civil Liability. After a recent report that permanent brain damage followed immunisation against whooping cough in one child in about 5,000, Mr Jack Ashley, a Labour Member of Parliament, has drawn attention to this anomaly in the law and has accused the government of "shattering complacency" in its attitude towards children damaged by vaccines.

A successful campaigner for compensation on behalf of Thalidomide children, Mr Ashley referred to a "veil of obscurity" shrouding the fate of thousands of children and he asked for urgent action to reduce the number of future tragedies. He has also proposed that compensation should be offered to those already severely damaged.

Following Mr Ashley's campaign in the House of Commons, an association formed last year in the interests of children damaged by vaccines has been receiving letters of support from parents.

The Association of Parents for Vaccine-damaged Children feels that Britain should have compensation schemes for the casualties of vaccine administration, as do most European countries. There

are no compulsory immunisation procedures in Britain, whereas they are common in the rest of Europe.

Compensation for damaged children was discussed almost a year ago at a conference held in Monte Carlo on vaccination against communicable diseases. Although there was a strong call for a code of guidelines for vaccine trials, it was pointed out that public concern would jeopardise future trials. Standards of conduct satisfying both scientific integrity and lay opinion would be required.

Another view expressed at the conference was that there is a good case for compulsory immunisation against some illnesses; for instance a child with quadriplegia due to poliomyelitis might be justified in suing his parents for negligence in not having had him immunised against the disease.

The compensation schemes practised in Denmark and Germany are controlled by the authorities, who are responsible for individual claims for damages. This means that if it is suspected that a vaccine is faulty the manufacturer can be sued.

The choice of vaccines used in European countries, including those for compulsory immunisation, differs widely and it is predicted that within the next few years closer agreement will be reached on the pattern of immunisation schedules.

In Britain concern is being expressed about the whooping cough vaccine which was first introduced nationally in 1957. Since then there has been a marked decline in the disease. It is, however, known that some children—there are no reliable figures—have been seriously damaged by the use of the vaccine. In 1972 general medical practitioners were given advice on possible adverse reactions to whooping cough vaccine and certain children were recommended not to receive it.

The Committee on Safety of Medicines was not entirely satisfied with the voluntary reports received on adverse reactions. The reports did, however, identify possible problems with adverse reactions in the various immunisation procedures. In fact neither the committee nor its predecessor, the Committee on Safety of Drugs received reports of direct adverse reactions to whooping cough vaccine.

Whooping cough vaccine is usually given as part of a triple injection for diphtheria, whooping cough and tetanus, or as a quadruple injection in which case poliomyelitis is the additional vaccine. Even though it is usually considered not possible to ascribe any adverse reactions to an individual vaccine the committee had received 503 reports of adverse reactions which included whoop-

ing cough vaccine; most reactions were however, minor.

In 1963 the number of notified cases of whooping cough started to increase again and have fluctuated about a number roughly one-fifth of the average in the decade before 1957. A survey carried out in 1969 on the whooping cough vaccines in use until 1968 suggested that although there was a marked decline in the disease, the vaccine had not been absolutely effective overall. The findings of the report suggested that in future the efficacy of the whooping cough vaccine should be kept under constant surveillance.

Since there are children definitely suffering from the effects of vaccines it would seem proper that the matter should be opened up and accurate figures revealed to substantiate the accusations now being made.

When Mr Michael Allison, Under Secretary for Health and Social Security, was questioned in Parliament on the success of immunisation he said that it could be measured by the virtual eradication of diphtheria and poliomyelitis. He also claimed that no immunisation procedure is entirely free from risk of ill-effects, even though he understood that about 170 reports of adverse reactions to vaccines of all types were received each year.

Mr Jack Ashley was not satisfied with Mr Allison's remarks and intends to submit a motion to Parliament calling for action on the cases of children harmed by administration of vaccines.

Prescribing safely for children

Fiona Selkink

SHOULD potentially dangerous drugs be prescribed for children under the age of 10, in the hope that they may alleviate some relatively trivial complaint of childhood? This is a question often raised and highlighted once again by a recent report in the *British Medical Journal* (BMJ) (1, 261; 1974).

The report is the results of a seven and one-half year survey of the cases of accidental drug poisoning in children admitted to the Royal Hospital for Sick Children in Glasgow (carried out by Drs K. M. Goel and R. A. Shanks). Of the 60 children suffering from poisoning by one or other of two drugs (amitriptyline or imipramine), three-quarters were between the ages of two and four years, more than half were poisoned as a result of having taken an excess of the drugs originally prescribed for themselves or for brothers and sisters. Of these 60 children, 30 were admitted during the past 18 months, indicating that this situation is worsening. The remaining 43% of children suffering from the

effects of poisoning had swallowed the same drugs prescribed for adults in the family.

The drugs in question are tricyclic hydrochlorides, commonly prescribed as anti-depressants in adults, and have been found to have varying degrees of usefulness in the treatment of nocturnal enuresis (bed-wetting) in children.

There is no positive information about the toxicity of these drugs in abnormal quantities. There is no known antidote. Some people believe that serious manifestations invariably appear after ingestion but others believe that the severity of poisoning is related to individual tolerance and that the severity of poisoning should be assessed symptomatically.

Any case of accidental overdose of tricyclics in children is treated with close observation for 24 hours and the experience of the Glasgow group is that if the child survives that first critical period, it will probably recover completely, with no after effects. The symptoms presented are treated where possible. The heart is restarted mechanically; the lungs ventilated; convulsions treated with drugs and the contents of the stomach are always removed.

Opinions vary as to whether the use of these drugs has any lasting effect on the condition. Dr Shanks firmly believes that they do not. He feels that at best the effects are only transient and that it seems that as soon as treatment is stopped the situation reverts to the *status quo ante*. A general medical practitioner who has prescribed these drugs from time to time for his patients, and has found their effect "quite impressive", says the limiting factor to the duration of treatment is usually the patient. Either he comes back for a repeat prescription because "the tablets you gave him last time worked so well doctor" or he stops coming. The doctor admitted however, that he knew cases where the condition seemed to have cleared, treatment had stopped and then, perhaps six months later, the patient's mother was back in the surgery, asking for more, because the child had started being wet again.

This doctor does not believe in treating children of pre-school age but older children, who are likely to suffer socially from the effects of this condition, he believes should get whatever help he can give them. Parents should be given something positive to do, so that they feel they are being of some help to the child also.

Having been quite impressed by the effectiveness of these drugs in the treatment of enuresis in his patients, the doctor said that he was "pretty horrified" when he read of the effects that accidental poisoning seem to produce in

young children.

On the whole, doctors are at their own discretion as to what drugs to prescribe to which patients, and how much and how often the medicine should be administered. They have as guidelines the literature produced by the pharmaceutical companies marketing the drugs, reports in the medical and scientific literature, training courses at universities and colleges and, occasionally, directives from the Department of Health and Social Security, distributed through the local Executive Councils. There are many journals available free or on subscription—one such is produced by the Consumer Council—which give doctors information about medicines, contain advice on which drugs established for the treatment of one condition have a beneficial effect for a completely different disease. The pharmacologists then have to discover the reason for the link.

When doctors prescribe drugs, especially for children, they generally warn the patient of the dangers associated with the medicine and remind them of the necessity to keep all medicine out of reach of children. Dr Shanks says that when parents of children who had poisoned themselves with the drugs were told of the dangers, they were naturally very upset and many said that they had not realised just how dangerous they were. If the rationale for prescribing tricyclics for young children is that they will relieve the anxiety symptoms manifested by enuresis (and one accepts that there are occasions when their use is justified), then perhaps the warnings should be spelt out more fully.

There have always been claims for the effectiveness of some treatment for a particular disease or condition but, on the whole, the cures were not as dangerous as the illnesses. Today, perhaps, there is too much emphasis placed on a cure at all costs, or at least easing of symptoms, and the advances of the pharmaceutical industry, together with the pressures of society, are just a little too great on the doctor with the prescription pad and the distressed patient.

Congress to lose key nuclear advocates

Colin Norman, Washington

THE Joint Committee on Atomic Energy, which for years has held a tight rein on legislation dealing with all aspects of nuclear energy in the United States, is about to lose two of its most powerful, and certainly its most colourful, members. Last month, Craig Hosmer, the most senior House Republican on the committee, announced that he will be retiring from Congress at the end of the year. And last week, his comrade-

in-arms, Chet Holifield, announced that he, too, will quit national politics this year after a 32-year stint in Congress. Their departure is raising some speculation about the future for the joint committee.

Holifield, a crusty Democrat who was a charter member of the committee and its chairman in alternate Congresses between 1963 and 1971 (the chairmanship alternates between a Senator and a Congressman), has decided to step down rather than face a tough challenge in a primary election in his California constituency. In 1971, he became chairman of the House Committee on Government Operations, a move which put him in a strong position to intercept and reshape the Administration's proposals for government reorganisation, and his Joint Committee mantle then fell to Melvin Price, a Democrat from Illinois.

Hosmer, Price and Holifield between them have largely dominated the work of the joint committee since the late 1950s. They have devoted their time almost exclusively to the committee's work, often at the expense of other activities, and this has given them a depth of knowledge which their opponents find difficult to overcome. All three are strong advocates of nuclear power, and they have been constantly attacked by environmentalists in the past few years.

Thus, with two members of the troika about to depart, a leadership vacuum is likely to emerge in the committee. Whether that will diminish the committee's power on Capitol Hill remains to be seen, but other developments are likely to have a more important impact on its future role. In any case, the unique status of the committee makes it an extremely difficult body to get rid of, or even to change.

The committee derives much of its power from several factors. First, the Atomic Energy Commission has a legal responsibility to keep the committee "fully and currently informed" of its activities, a requirement which gives committee members complete access to the agency's plans and deliberations. Ralph Nader recently remarked in a hearing before the joint committee that a result of this requirement is that the committee "has acted more like a part of the executive branch than any other committee of Congress", and it is often accused of being a mouthpiece for, rather than a watchdog of, the Atomic Energy Commission.

Second, it is the only legislative committee of Congress which has responsibility for nuclear energy. Other matters are considered by separate committees in the House and the Senate, and interchange between committees is frequently important for bringing out an resolving important problems. According to the

joint committee's critics, the lack of interchange on nuclear energy matters often has the result that differences of opinion fail to surface in Congress.

And third, the committee was established by an Act of Congress, and thus its functions and structure cannot be altered unless Congress passes a bill to do so. Since such a bill would be referred to the Joint Committee, its future is entirely in its own hands.

One development could, however, have a profound impact on the committee. Congress is now considering a bill to create an Energy Research and Development Administration (ERDA), based on the laboratories of the Atomic Energy Commission, but including energy research and development programmes from other agencies. If Congress passes the bill, the AEC would be split in two—the regulatory functions would be put into a separate agency—and the joint committee would be left with authority over an agency with which many other committees would also be concerned. A bill to establish ERDA has already been approved by the House of Representatives, and is now under consideration by the Senate Government Operations Committee.

Whatever the prognosis for the ERDA bill, the joint committee will certainly be a different body without Hosmer and Holifield. Their hearing room style alone, which ranged from bitingly sarcastic to gentle bullying—but which was always incisive—will be especially missed from the committee's deliberation.

Hearing double

A CURIOUS piece of programming by the BBC on Monday February 11 produced "Scientifically Speaking" on Radio 3 commencing at 2125, and "Kaleidoscope" on Radio 4 commencing at 2130. As if this were not enough to strain credulity, listeners with two radios were then treated to a simultaneous discussion of black holes and X-ray sources, with special reference to Cygnus X-1, on each channel.

On Radio 4 Professor R. L. F. Boyd could be heard confounding the interviewer (who may have been intimidated by the knowledge that Professor Boyd is a member of the BBC's Science Consultative Group) with a moderately hairy description of the black hole phenomenon which made no concessions to the level of scientific interest of the "Kaleidoscope" audience; on the theoretically more highbrow Radio 3, the lucid discussion by Professors Martin Rees and Ken Pounds was, by contrast, so clear that confused "Kaleidoscope" listeners who switched channels hoping to catch the end of a concert might even have stayed for the end of the programme.

correspondence

Is anonymity necessary?

SIR,—As co-editor of a series of books (*Comprehensive Virology*, Plenum, 1974) I was recently taken to task concerning the advisability of anonymous reviewing of solicited chapters. We fully agree with this criticism and will in the future request our reviewers to submit their constructive critical comments over their signature, so that the author can ask for clarification or defend his position.

As a past or present member of the editorial boards of several journals as well as frequent reviewer and reviewee of papers, I would like to broaden this enquiry into the effect of anonymity in the domain of reviewing for scientific journals. I was recently asked to review, and advocated rejection of, a paper for a virological journal on the basis of factual comments which I would have been quite willing to sign. The editor sent me, out of courtesy, copies of his rejection letter together with the other reviewer's sarcastic poison-pen comments, also rejecting the paper. There was no justification for one civilised person insulting another in such manner (as well as a specified alternative "boring" journal that might be willing to publish such "routine and dull work"); that outburst was solely the joy of releasing adrenaline with anonymous impunity.

I have read many other reviews which were full of bias. In one recent instance the reviewer, not wishing to accept the well proven conclusion of the author, misquoted the literature to prove his point to an editor who would not have the time to verify his citations.

I have given this matter considerable thought. There is no question that much good comes out of careful and conscientious reviewing and that scientific communication would not be improved by abolishing it altogether. But anonymity tends to bring out the worst in people and it causes undesirable aspects of the reviewing system which I believe to be unnecessary. Thus I advocate that editorial boards could and should come out strongly in favour of reviewers identifying themselves when they detect factual errors, weaknesses in the argument, inadequate or erroneous citations, or lack of clarity in a manuscript. The author can then consider these comments in the same polite and considerate style that such reviews would show.

This suggestion does not preclude the reviewer expressing his conclusions di-

rectly to the editors if he feels this to be necessary or helpful. If he advocates rejection in his letter to the editor, and if this is supported by the factual arguments given to the authors which in turn are not easily and clearly countered by them, then the scientific level of the journal would not be diminished, and the state of mind of the scientific community would be noticeably improved.

Yours faithfully

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Protein Gap

SIR,—The Food and Agriculture Organisation of the United Nations and the World Health Organisation have just published jointly their latest report on energy and protein requirements of man based on a comprehensive review of 50 years research in the field. A comparison of the average requirements per person based on this report¹ with average intakes shows that there is no protein gap at the national level. In most countries the average protein intake exceeds the average requirement so considerably that inequality in the consumption also fails to explain the full extent of the incidence of protein malnutrition. Furthermore, in those few individuals whose protein intake is found to be inadequate, the deficiency of energy intake relative to requirement is even more marked. Clearly, what diets lack is not protein but energy to metabolise the protein people actually eat^{2,3} (Miller and Payne, 1969; Sukhatme, 1969). In the circumstances the United Nations call to intensify production of semi-conventional protein-rich foods for closing the so-called protein gap seems to have little to commend it to the developing countries (U.N. General Assembly Resolution 1970).

Surprisingly, however, the 1973 report incorporates a new interpretation of the meaning of protein requirement to help planners evaluate an apparently non-existent protein gap. This interpretation is that an individual eating below the recommended level, called a 'safe' level in the report (that is the average requirement plus twice the standard deviation), while not necessarily malnourished, runs the risk of developing protein deficiency and that the risk increases as the intake falls below the "safe" level.

The implication is that individuals should be counselled to eat at levels above the "safe" level and that countries should aim to increase their protein supplies to make this possible. Calculations based on the normal bivariate distribution model for intake and requirement, which is implicit in the new interpretation of requirement show that the needed increases are very large; so large in fact as to be considered "unrealistic of the situation in the developing countries"⁴. In effect this exercise implies that 97.5% of the population would have to eat at levels of overconsumption—or waste—several times greater than the average needs to limit the population at risk (that is below the "safe" level) to 2.5%. If it were not protein but some other nutrient, say Vitamin A, these levels would be in the toxic range!

Evidently the assumptions implicit in the new interpretation of requirement require careful evaluation. I have examined them and find that they are not supported by available data. In particular, I find that the basic assumption that the requirement of an individual, like his intake, is known and can be determined with an error of measurement which can be assumed to be negligible, is not borne out by the data. I find that intra-individual variability in requirement often accounts for a major part of the total variation. Consequently neglecting to take it into account gives a grossly exaggerated and misleading picture of the protein problem in the countries concerned. In a similar vein, such statements as "two-thirds of the world is hungry and malnourished" as was first suggested by Lord Boyd Orr and which continue to be repeated in evaluating the nutritional situation in the developing countries have their origin primarily in the failure to recognise the status of the variable quantified by requirement. Details will be found in Sukhatme^{5,6}.

Yours faithfully,

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¹ WHO, *Energy and Protein Requirements* 522, (Geneva, 1973).

² Miller, D. S., and Payne, P. R., *Proc. Nutr. Soc.*, **28**, 225 (1969).

³ Sukhatme, P. V., *Indian J. med. Res. New Delhi*, **57**, 2170 (1969).

⁴ Beaton, G. H., *Proc. Western Hemisphere Natl. Congress*, (Miami, 1972).

⁵ Sukhatme, P. V., *J. Roy. Stat. Soc. Series A* (in the press).

⁶ Sukhatme, P. V., *Proc. Nutr. Society*, **33**, 36A (1974).

news and views

Have tachyons been observed?

ONCE upon a time discoveries in physics were made by experimentalists who used their skills to detect the new and the unexpected. Interpretation came later. Nowadays, however, it is as often as not the theorist who takes the initiative. An existing theoretical framework may be extended in a self-consistent way that leads to quite specific predictions concerning new effects. The experimentalist is then invited to devise an experiment that will 'verify' or 'confirm' the predictions. Elementary particle physics, in particular, illustrates this trend. The pion and the Ω^- particle are famous examples of successful predictions. Particles that remain on the 'wanted' list include the legendary quarks—particles of large mass and fractional charge—and the tachyons. The quarks, if they exist, would provide a neat interpretation of the regularities observed among the known elementary particles (SU3 symmetry). The tachyons would allow one to extend the ideas of relativity into the region where velocities greater than the velocity of light occur.

In the usual interpretation of relativity, it is stated that particle velocities v can never exceed the velocity of light c . The reason is that the total energy $mc^2[1 - (v/c)^2]^{-1/2}$ must be a real quantity. To obtain a real value for the energy when $v > c$ we must suppose that the particle concerned is one for which the rest mass parameter m_0 is not a real number. Instead we must put $m_0 = im_1$ with m_1 real. If this extension to the theory is physically valid, then tachyons can exist and their total energy is given by $m_1c^2[(v/c)^2 - 1]^{-1/2}$. It follows that tachyon velocities are restricted to the range $v > c$ and that their energy diminishes as v increases. In other words, energy loss produces acceleration. Other, somewhat bizarre, properties can be expected. A charged tachyon, for instance, automatically fulfils the conditions for emission of Čerenkov light, even in free space. It must therefore radiate energy spontaneously and suffer continuous acceleration to higher and higher velocities. It has no possibility of 'uniform motion in a straight line'.

The experimental search for tachyons is based on the supposition that they can be produced during elementary particle interactions and can subsequently cause such interactions. Some investigators have performed bubble chamber plus accelerator experiments; others have made use of the cosmic-ray flux and have looked for tachyon production in the atmosphere. The bubble chamber studies apply the requirements of energy-momentum conservation to obtain the square of the mass of an unknown particle produced in the interaction. This is standard practice. For ordinary particles, the (mass)² is, of course, positive but for tachyons the value would be negative. So far, this search has been unsuccessful. The cosmic-ray search depends on the possibility of tachyons being produced in the early stages of development of extensive air showers. Since the tachyons travel (by definition) faster than c , they must reach the ground before the shower itself. If they are capable of producing a response in ordinary particle detectors, one should therefore observe particles associated in time with the air shower but occurring some tens of microseconds earlier.

An experiment of this kind is described by Clay and

Crouch in a communication on page 28 of this issue of *Nature*. With due caution, after a careful statistical analysis of their measurements, they conclude that they have indeed "observed non-random events preceding the arrival of an extensive air shower". This result should encourage others to continue the search. Until the measurements provide something more tangible in the way of estimated cross sections and other limitations on the parameters used to describe the particle, it is unlikely, however, that they will inspire more than polite interest among theoretical physicists.

From a Correspondent

Structure of transfer RNA

NUCLEIC acid research entered a new era, when a little more than a year ago, Kim, Rich and their colleagues at the Massachusetts Institute of Technology, reported their success in the X-ray analysis of a crystalline transfer RNA. Their studies, at successively 5.5Å and 4.0Å resolution (*Proc. natn. Acad. Sci. U.S.A.*, **69**, 3746; 1972; and *Science*, **179**, 285; 1973), enabled the overall shape of the molecule to be determined with a fair degree of certainty. They now report, on page 20 of this issue of *Nature*, their latest results at an increased resolution of 3Å, and their new electron density maps reveal considerably more detail than hitherto. Thus, whereas at 4Å, the ribose-phosphate groups were discernible as individual peaks, the higher resolution data have enabled these to be resolved into two separate peaks, in many instances with a consequent increase in the confidence of the electron density interpretation.

The dominant feature of the 4Å model of Kim *et al.*, in which the molecule has an overall L shape, has been amply confirmed in the new electron density maps. The course of the polynucleotide chain was followed by tracing the characteristic ribose-phosphate zig-zag which was clearly seen in many places, especially double-helical regions. Base pairs also can be easily observed in many regions, and the double-helical parts of the molecule are all well resolved, although one base cannot be distinguished from another. Difficulties were evidently encountered, however, when they tried to follow the course of the chain in the non-helical regions of the molecule—the so-called loop segments. These seemed to be due partly to chain irregularities, and also to ambiguities in the maps in these regions arising from extraneous peaks which the authors ascribe to bound cations. The two loops, pseudouridine and dihydrouracil, which are somewhat crowded together close to the corner of the L, suffer especially from these uncertainties. Likewise, the CpCpA acceptor stem of the tRNA molecule is not well defined, though this may be a consequence of the inherent flexibility and sloppy nature of this grouping.

Several other details that were not apparent in the earlier low resolution studies have emerged. Previously the molecule was seen to have the two arms of the L consisting of two roughly perpendicular double-helical branches, each of which consisted of two colinear stems. This picture has had to be modified so that now only one branch has colinearity, the other has the anticodon and the dihydrouracil stems mutually inclined at an angle which may be as much as 30°.

It is perhaps not too surprising, in view of their shortness, that none of the four helical stems has been found to be quite regular, and that they cannot be described in terms of exact helical parameters. The authors have also been able to distinguish various other interesting features of these stem regions; thus, purine bases at the end of the helices often lie in stacked arrangement with respect to them. Similarly, the G-U base pair which lies in the middle of the CpCpA stem is also stacked parallel to the other double-helical bases in this region. The anticodon loop of the tRNA molecule, which is involved in coding recognition during protein synthesis, is seen to have the bases on the outside of the loop, although, again, there are uncertainties here.

It is unfortunate that the features of tRNA that are of greatest functional interest are so far poorly resolved. The MIT workers are now extending their analysis to include all the reflections occurring in the diffraction patterns, which extend to a resolution of 2.3 Å. It is to be hoped that this work, together with additional heavy-atom derivatives to improve the phasing, will result in a good definition of the complete molecular structure.

The next stage in tRNA crystallography—the structural analysis of species other than the yeast phenylalanine tRNA of Kim *et al.*—still seems some way off. Only then will one really be able to discuss the relations of tertiary structure to biological function by correlating structural features common between tRNAs. S.N.

Towards a more efficient use of cytotoxic agents

BIOLOGISTS who seek, by the introduction of defined chemical agents into the body of man to alleviate, remedy or cure its many ailments, have a peculiarly mixed credo. There are the “eye of newt and toe of frog” experts (*Macbeth*, Act IV, Scene 1) who are happy if they can produce a relatively effective agent which does not have any apparent and immediate side effects. Others, pursuing the notion of “sufficient unto the day is the evil thereof” (*St Matthew*, VI, 34) have devised agents of known toxicity which despite their side effects and lack of specificity are nevertheless used as the only ones available. In a situation where there are very few medicaments whose effects can be thoroughly understood, it is reasonable that the art of expediency should have been practised. On page 82 of this issue of *Nature* appears an article by Rubens and Dulbecco which is one of a series of attempts to provide a more rational basis for chemotherapy—in this instance of cancer.

Many cytotoxic agents have been used to attack cancer cells. In all instances there have been undesirable side effects on vital organs such as bone marrow so that the clinician has always had to consider the balance between desirable tumour cell eradication and potentially lethal toxicity. Attempts have been made to improve the specificity of action of these agents so that their cytotoxicity is concentrated in malignant cells. Ingeniously prepared ‘latently active’ agents which are themselves quite non-toxic but which can transform to toxic products selectively in tumour cells have been synthesised. Another approach has been to use larger molecular weight materials as carriers of the cytotoxic chemicals. Daunorubicin, for example, is more effective against the L1210 leukaemia in mice if it is administered as its intercalated complex with DNA (Trouet, Deprez-de-Campeneere and de Duve, *Nature new Biol.*, **239**, 110; 1972). The complex seems to be taken up selectively by tumour cell endocytosis. Inside the cell the DNA of the complex is digested by lysosomal enzymes and the daunorubicin is released in its active form and attacks nuclear DNA.

The results in the laboratory have been of sufficient interest to lead to a clinical trial of the complex in the treatment of myeloid leukaemia (Sokal, Trouet, Michaux and Cornu, *Eur. J. Cancer*, **9**, 391; 1973). Proteins have also been used as carriers of anti-cancer agents and albumin has been shown to be selectively incorporated into tumour areas (Busch and Greene, *Yale J. Biol. Med.*, **27**, 339; 1955). Similarly, fibrinogen seems to concentrate in the necrotic regions of tumours (Islaker, Cerottini, Jaton and Magnenat, *Chemotherapy of Cancer*, edit by Plattner, Elsevier, Amsterdam, 1964) and attempts have been made locally to irradiate cancers by the use of radioactive iodinated fibrinogen and anti-fibrin antibody. Globulins, fibrinogens and albumins have been covalently bound to alkylating agents and antimetabolites in a further attempt to concentrate the cytotoxic agent in tumour cells (Wade, Whisson, and Szekerke, *Nature*, **215**, 1303; 1967; Mathe, BaLoc and Barnard, *C. r. hebd. Séanc. Acad. Sci., Paris*, **246**, 1626; 1958; Larsen, *Eur. J. Cancer*, **3**, 163; 1967). In some instances covalent attachment to protein has increased the selectivity of action of the anti-tumour agent. One interesting finding was that nitrogen mustards merely absorbed (and not covalently bound) to protein could also be more selective than the mustard alone (Wade *et al.*, *loc. cit.*).

An extension of this work and one more likely to give greater specificity has been to attach cytotoxic agents to tumour specific antibodies. Alkylating agents conjugated to antibody directed against tumour cell antigens have been quite effective (Ghose, Cerini, Carter and Nairn, *Brit. Med. J.*, **1**, 90; 1967), but more surprising has been the finding that the alkylating agent chlorambucil merely absorbed to an immunoglobulin fraction is a much better inhibitor of an experimental tumour than either chlorambucil alone or absorbed to a normal globulin fraction (Ghose, Norvell, Guclu, Cameron, Bodurtha and MacDonald, *Brit. Med. J.*, **3**, 495; 1972). This is surprising since the absorbed alkylating agent would be expected to dissociate from the globulins on injection. The basis for the improved selectivity is thus not at all clear, but the results have been confirmed by the related work of Flechner (*Eur. J. Cancer*, **9**, 741; 1973) who showed that anti-Ehrlich ascites globulin complexed with chlorambucil could cure mice bearing the Ehrlich ascites tumour.

In an attempt to explain this phenomenon, Rubens and Dulbecco (this issue) have shown that chlorambucil and a tumour specific globulin are indeed synergistic but that there is no requirement for physical absorption between the two since separate addition of the two components can still bring about the desired effect.

Obviously the cytotoxic agent and the tumour specific globulin act together, but not by the protein acting as a carrier. The mechanism once elucidated may lead to more efficient use of the cytotoxic agents in the treatment of cancer.

T.C.
A.D.

C-type viruses of primates

BIOLOGISTS who hanker after new species of organisms may profitably examine primates for hitherto undiscovered viruses. In recent years more than twenty new herpesviruses have been described, and now several groups under contract with the Virus Cancer Program of the US National Cancer Institute have detected RNA tumour viruses in normal or malignant tissues of primates.

The first virus, known as Mason-Pfizer virus, was isolated by Chopra and Mason (*Cancer Res.*, **30**, 2081; 1970) from a mammary tumour of a Rhesus monkey. The Mason-Pfizer virus has a morphology intermediate between the B-type

(mammary tumour viruses) and C-type (leukaemia-sarcoma viruses) characteristic of mice and other vertebrates. Then a C-type virus was isolated from a sarcoma of a woolly monkey by the comparative oncology group at the University of California at Davis (Theilen *et al.*, *J. natn. Cancer Inst.*, **47**, 881; 1971) and this virus was found to induce sarcomas when inoculated into marmosets (Wolfe *et al.*, *J. natn. Cancer Inst.*, **47**, 1115; 1971).

The Davis researchers also isolated a C-type virus from a lymphosarcoma of an ape, the white-handed gibbon (Kawakami *et al.*, *Nature new Biol.*, **235**, 170; 1972). Several lymphomas and leukaemias of gibbons have been reported lately, and C-type particles have also been observed in the only other one examined (Snyder *et al.*, *J. natn. Cancer Inst.*, **51**, 89; 1973). These observations, however, apply to gibbons which have been kept in captivity for several years and some have been subjected to experimental procedures such as X irradiation which may have rendered those animals susceptible to unusual infections. Immunological and nucleic acid hybridisation studies indicate that the viruses isolated from the woolly monkey and the gibbon are very closely related. Considering how widely separated in evolution and geographical distribution are the New World monkey and the ape, it seems possible that at least one of the C-type viruses has been acquired in captivity from another source, rather than that they represent natural infections for both species.

Three recent reports describe C-type virus particles associated with primate placental tissues. Schidlovsky and Ahmed of Pfizer Inc. (*J. natn. Cancer Inst.*, **51**, 225; 1973) observed particles by electron microscopy budding from the syncytial trophoblast in each of four Rhesus monkey placentae and also in erythroblasts of foetal haemopoietic tissues. Kalter and his colleagues at the Southwest Foundation for Research and Education, Texas (*Science*, **179**, 1332; 1973) independently observed C-type particles associated with the placenta of baboons. The virus particles were prevalent at the syncytial trophoblast of each of thirteen normal baboon placentae at various stages of gestation. These findings naturally led Kalter and his colleagues to investigate human tissue (*J. natn. Cancer Inst.*, **50**, 1081; 1973) in which they also claim to see C-type particles, though very few in number, in four of six human placentae. Each placenta required several hours of screening with the electron microscope before particles plausible enough for publication were photographed.

Kalter generously provided a baboon placenta to Todaro's laboratory at the

US National Cancer Institute and the isolation in infectious C-type virus is reported in this issue of *Nature* (page 17). The placental tissue was cocultivated with various mammalian cell lines and virus was found to replicate most efficiently in a canine thymus cell line. Molecular hybridisation studies using complementary DNA transcribed from the RNA of the propagated virus do not indicate any homology between the baboon virus and other C-type viruses, including those isolated from the woolly monkey and gibbon. There is, however, substantial homology with DNA extracted from normal baboon placenta and liver and with RNA extracted from the placenta.

These results suggest that the C-type virus represents an endogenous, vertically-transmitted virus of baboons, that is normally expressed in the syncytial trophoblast but not in most other tissues. No doubt several laboratories are now busy cocultivating human placental tissue with every mammalian cell line that comes to hand in the hope of propagating an endogenous human C-type virus. They may well be successful.

From a Correspondent

Replication in synchronised cells

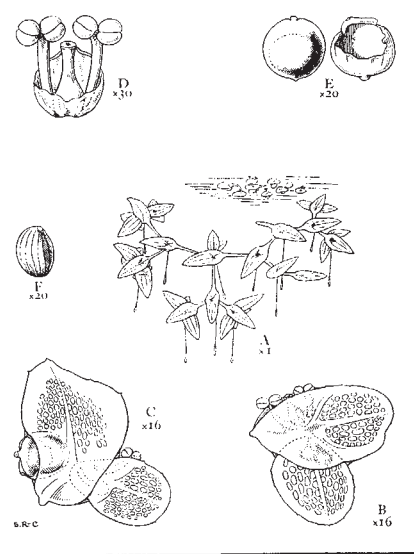
from a Correspondent

Non-random DNA replication during S phase of cultivated cells has been known since the early days of autoradiography. Moreover, analysis of the replication time of easily purified specific DNA sequences, such as the nuclear satellite DNAs found in many species of animals and plants, suggests that the ordering of replication is regulated by the DNA sequences involved. Although the exact point of replication of a particular DNA sequence may depend on the physiological state of the cell—for instance normally mouse satellite DNA replicates late in S phase but it seems to replicate in early S phase in cells infected with polyoma virus (Smith, *J. molec. Biol.*, **47**, 101; 1970)—it seems to be constant from one cell generation to the next.

Several descriptions exist for the replication time of the multiple genes for ribosomal RNA but they may be criticised on two grounds; one is lack of sensitivity of the technique used for assaying rDNA and the other is the inadequacy of the technique used for synchronising the cells. In a recent issue of the *Journal of Molecular Biology* (**82**, 303; 1974) Stambrook reinvestigates the temporal replication of ribosomal genes. He used Chinese hamster ovary cells synchronised by the 'wash off' procedure for collecting cells in meta-

Last of the line

THE publication of part XXXI of Stella Ross-Craig's *Drawings of British Plants* (Bell, 1973) brings to an end a series which started life in 1948 with Ranunculaceae and which has covered all British flowering plants except the Cyperaceae and the Graminae which have been illustrated adequately elsewhere. Lemnaceae (duckweed), Alismataceae (water plantain), Juncaginaceae (arrow grass), Potamogetonaceae (pondweed) and Zosteraceae (grass wrack) are among the families included in the last part. This figure shows the form of the ivy-leaved duckweed *Lemna trisulca*.



phase together with a highly sensitive assay for newly synthesised rDNA. Synchronised cells were labelled with bromodeoxyuridine (BUdR) for successive intervals of 2 h covering the 8 h duration of S phase and the DNA was isolated. Newly replicated DNA will be labelled with BUdR in one strand and will band in a neutral CsCl gradient at a greater density than the unreplicated DNA. Following banding in CsCl gradients, DNA fractions were denatured, loaded on to filters and challenged with a very high specific activity complementary RNA made *in vitro* using purified *Xenopus* rDNA and *Escherichia coli* RNA polymerase. The proportion of rDNA replicating during the BUdR pulse is equal to the proportion of cRNA hybridising to high density DNA, and the proportion of total DNA replicating during the same 2 h is given by the proportion of total optical density banding at high density. Although the nucleic acid hybridisation reaction is heterologous, Stambrook found that it has high fidelity. The cRNA is competed to a high level with Chinese hamster rRNA and also hybridises to a high density

component of total DNA in the same position as authentic ^3H -labelled Chinese hamster RNA.

Stambrook found that approximately 50% of rDNA replicates during the first 2 h of S phase during which time only 27% of the bulk DNA replicates; most of the remaining rDNA, together with 36% of the bulk DNA, replicates during the next 2 h. Studies with other mammalian cell systems have given very different results, for instance HeLa cells replicated ribosomal genes during the whole of S phase (Balazs and Schildkraut, *J. molec. Biol.*, **57**, 153; 1971) and rat kangaroo cells replicated them at the end of S phase (Giacanami and Finbel, *J. molec. Biol.*, **70**, 725; 1972). It is possible that the lack of a discrete replication time in HeLa cells results from a breakdown of some regulatory mechanism which coordinates the replication of rDNA and which is responsible for the apparent synchrony of replication of rDNA in other cells. A more likely explanation, since the multiple ribosomal genes in HeLa cells are localised on five separate chromosomes whereas those of Chinese hamster and rat kangaroo are probably located on one chromosome, may be that coordination of replication is related to locus, separate loci being under independent control. Although against this interpretation is the observation that satellite DNAs are spread over several chromosomes and replicate together at the end of S phase.

One criticism of Stambrook's experiments is that there is no indication of the molecular weight of DNA used in the CsCl gradients. It could be argued, since ribosomal RNA coding sequences are known to be intermingled with spacer DNAs of approximately equal length, that early replication of spacer DNA in the absence of replication of ribosomal RNA coding sequence would lead to the latter banding at higher density. Banding of DNAs with molecular weights less than one complete rDNA repeat unit (8×10^6 daltons) should in principle distinguish this possibility.

Stimulation of tumour cells by antibody

from a Correspondent

THE ways and means by which the immune system elects the destruction of tumour cells have always held centre stage in tumour immunology, but a gentle push towards the wings now seems to be in the making. That push is being exerted by immunologists who maintain that the immune response, in addition to its ability to inhibit tumour growth, can—in the appropriate conditions—

stimulate it. This immunostimulation theory was first put forward by Prehn a few years ago, the essence of it being that a weak immune response could encourage growth of nascent tumours, whereas a more potent immune response could effect the opposite result.

How this effect might occur is the subject of a paper by Shearer, Philpott and Parker in a recent issue of *Science* (**182**, 1357; 1973); the authors studied the effect of adding specific antisera to various cultures of human or mouse tumour cell lines. In all cases it was found that the addition of small concentrations of antibody brought about enhanced cell growth, as assessed by increased cellular incorporation of radioactive precursors of nucleic acids. Conversely, high concentrations of antibody inhibited cell growth, effects which clearly mimic Prehn's earlier *in vivo* studies on the effect of transferring high or low numbers of immune lymphocytes along with tumour cells into a non-immune host (*Science*, N.Y., **176**, 170; 1972). Furthermore, the authors found that the effect could be duplicated by coupling a chemical haptenic group to the tumour cells and subsequently adding various amounts of antibody with specificity for the chemical grouping to the cell cultures.

These results, taken together with Prehn's, clearly indicate that antibody molecules against tumour cell surface components can induce alterations in growth behaviour which are strikingly similar to those seen when lymphocytes are used as target cells, and imply yet another mechanism by which antibodies can enhance tumour growth.

An afterthought of these experiments is whether antibody of irrelevant specificity, if it can be made to interact with a tumour cell, will induce a similar kind of stimulatory effect. Such a union is made plausible by the surprising demonstration that a variety of non-lymphoid tumours in the human have receptors for antigen-antibody complexes (so-called Fc receptors) which recognise the Fc region of immunoglobulin molecules when complexed to specific antigen (Tønder and Thunold, *Scand. J. Immunol.*, **2**, 207; 1973). Such receptors have been described as an array of cell types including macrophages, mast cells, granulocytes, neutrophils and lymphocytes. Furthermore, it is well known that immune complexes can stimulate cell division of lymphocytes *in vitro*. Considering the similarity of certain populations of tumour cells and lymphocytes with regard to the effects that specific antibody on their growth characteristics, as well as the presence of Fc receptors, it might not be too surprising to find that certain kinds of immune complexes can stimulate or inhibit tumour cell growth.

Do mantle plumes produce copper?

from our Geomagnetism Correspondent

ALTHOUGH the various hypotheses making up the new global tectonics have combined to form a viable framework for the behaviour of the Earth as a whole, much still needs to be done in interpreting more local geological data in terms of the wider, and hopefully unifying, concepts. Indeed, the job of interpreting new data and reinterpreting the old is likely to be the main preoccupation of Earth scientists for many decades to come, not only in connection with 'pure' geological phenomena but also as a first step towards more successful methods of prospecting for mineral resources. But there are dangers, for in spite of the success of the new global tectonics, some of the basic concepts involved are still matters of dispute; and a too premature and uncritical application of them in more localised situations could easily lead geology, and especially economic geology, into a series of costly blind alleys.

The problem is well illustrated by Livingston's attempt (*Earth planet. Sci. Lett.*, **20**, 171; 1973) to reinterpret the formation of porphyry copper deposits in the south-western United States in terms of the activity of a mantle plume. The deposits in question are "geochemically distinctive petrological entities" containing anomalously high concentrations of copper, sulphur, molybdenum, gold, silver and rhenium, and are related genetically to the intrusive phases of intermediate to silicic volcanic complexes. Potassium-argon dating, for example, has shown that the igneous intrusion and the metallisation were very close in time. On the other hand, there are both barren and productive intrusions with similar lithophilic element compositions, which leads Livingston to suggest that the formation of those intrusives carrying metallic sulphides of commercial significance is governed by some "unique factor". This unique factor, he then claims, is a mantle hot spot, although this does not necessarily mean that the sulphur and metals are derived directly from the deep mantle.

The chief evidence in favour of metal formation by hot spot comes from the distribution of the deposits in space and time. All of the porphyry deposits considered by Livingston have potassium-argon ages in the range 52–72 million years, which is identical to the range of ages for the associated volcanic and intrusive rocks and co-incident with the time span of the classical Laramide orogeny. But when the ages of the individual deposits are

examined in greater detail, they are not found to be distributed completely randomly within the geographical area concerned. Although there is considerable overlap there is a definite trend of decreasing age to the south-east (actually S40°E), a pattern which Livingston concludes could not be due to slow cooling. Instead, it appears that the "source" of the deposits moved towards the south-east at a rate of $3.57 \pm 0.65 \text{ cm yr}^{-1}$ —a rate sufficiently typical of plate motions to suggest that such motions are responsible for it.

The first plate tectonic feature to come to mind in this connection is the postulated subduction zone off the Pacific coast of Baja California, but although this is of the right age it would be unlikely to produce deposits whose ages vary along a line parallel to it. The alternative is a magma source which either moves towards the south-east in the lithosphere or asthenosphere, or remains stationary in the asthenosphere or mesosphere as North America moves north-westerly above it in response to global tectonic forces. The latter, adopted by Livingston as the simplest hypothesis, is a mantle plume which, taking account of the areal spread of the copper deposits, had an apparent surface diameter of 450 km. As far as the existence of copper emplacements is concerned, it also had a rather limited life of about $20 \times 10^6 \text{ yr}$, although the discovery of further evidence extending its existence cannot yet be ruled out.

On the wider scale, Livingston admits that the direction of plate motion implied by his hot spot appears to be inconsistent with the motions postulated by Dietz and Holden (*J. geophys. Res.*, **75**, 4939; 1970) and Phillips and Forsyth (*Bull. geol. Soc. Am.*, **83**, 1579; 1972) but suggests that his data represent motional details not resolved by "the more generalised approach". More critically perhaps, there is an apparent inconsistency with the directions of North American plate motion postulated by McGregor and Krause (*J. geophys. Res.*, **77**, 2526; 1972) and others. McGregor and Krause, for instance, assuming the Corner Seamounts of the North Atlantic to be the result of a hot spot, concluded that part of the North Atlantic moved in the S65°W direction subsequent to $74 \times 10^6 \text{ yr}$ ago. In fact, Livingston is able to show that this motion and his data are consistent if during the relevant period the North American plate rotated in a clockwise direction at an average rate of 0.35° per 10^6 yr about a pole located at about 50°N, 64°W, although to the extent that these figures are based on only two supposed plume traces they are probably not too accurate. Finally, Livingston claims that

his hypothesis is consistent with the available palaeomagnetic and palaeoclimatological data.

It is all so plausible; yet at the same time it somehow lacks conviction. Livingston is certainly right to document the degree to which the relevant data are consistent with the hot spot hypothesis, but for the time being it should be remembered that the very existence of hot spots and mantle plumes is widely disputed. The hot spot/mantle plume, though the least widely accepted of the phenomena associated with the new global tectonics, may ultimately become a part of the geological consensus. In the meantime, however, the dangers of introducing more and more of them to account for more and more localised phenomena should not be overlooked.

Attaching to the membrane

from our
Molecular Genetics Correspondent

ANY idea that the chromosome of *Escherichia coli* might possess only an indistinct structure was dispelled by the report of Stonington and Pettijohn (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 6; 1971) that it can be recovered from lysed cells as a compact structure sedimenting very rapidly, and further rebutted by Worcel and Burgi (*J. molec. Biol.*, **71**, 127; 1972) who found that this structure is organised in a particular manner. Worcel and Burgi suggested a model in which the *E. coli* chromosome is highly folded, with from 12–80 loops each maintaining a supercoiled structure; the loops may be attached to a core of RNA. Variations in the size of the folded chromosome (from 1,300–2,200S) were attributed to differences in the amount of DNA present, this depending on its state of replication. In an extension of this work, Worcel and Burgi now turn their attention to the membrane attachment of the folded chromosome and its relationship with the replication of DNA in the cell cycle (*J. molec. Biol.*, **82**, 91; 1974).

Two types of folded chromosome particle can be obtained from *E. coli* cells depending on the conditions of lysis. At low (0–4°C) temperature, the released particles sediment at 3,000–4,000S, whereas at room temperature the folded chromosomes sediment at 1,300–2,200S. At intermediate temperatures, both types of particle are found in the same lysate and can so be directly compared. Examining these two types of particle, Worcel and Burgi found that the protein:DNA ratio is always some ten times greater in the heavier class; the amount of nascent RNA (corre-

sponding to the entire cellular content) remains the same. The dense particles appear to represent chromosomes attached to the membrane, whereas the lighter ones correspond to chromosomes not so attached. (Worcel and Burgi suggest the nomenclature "membrane-attached chromosomes" for the heavy preparation and "folded-chromosomes" for the lighter preparation.) Folded chromosomes can be released by solubilising the phospholipids of the membrane-attached chromosomes. Core RNA polymerase is the main protein in the released folded chromosomes, but the membrane-attached chromosomes have in addition a large content of membrane proteins.

Membrane-attached chromosomes cannot be obtained from cells which have been starved for amino acids (this treatment allows completion of current rounds of replication but prevents initiation of new rounds); all of the DNA from these cells sediments as the light form of the folded chromosome. But when leucine is added to the starved cells, the chromosomes appear to reattach to the membrane. Although there is a lag of 30 min before replication recommences after the addition of leucine, all the chromosomes become membrane-attached within 20 min. And when amino acid starvation is imposed during replication, chromosomes continuing DNA synthesis appear to remain attached to the membrane. Association of the folded chromosome with the membrane thus seems to be directly related to DNA replication.

Earlier experiments have, of course, suggested that the origin and replicating forks of bacterial DNA are enriched in membrane fractions. But there are technical difficulties which make qualifications necessary in the interpretation of almost all of these experiments. To provide a critical test of the hypothesis originally proposed by Brenner, Jacob and Cuzin in 1963 (*Cold Spring Harb. Symp. quant. Biol.*, **28**, 329), that the replicating chromosome of *E. coli* is attached to a site on the cell membrane and that attachment to this site could be the critical event which controls cycles of replication, therefore requires, as Worcel and Burgi point out, experiments utilising procedures which do not alter the properties of the membrane-DNA complex as the cell is lysed. Just such criteria seem to be met in these latest experiments.

The reason for the increased sedimentation velocity of the membrane-attached form of the chromosome is not known, but may be due to either or both of the extra mass of the membrane fragments attached to the DNA or its mode of folding. In a second article, Delius and Worcel report electron mi-

croscopy of the two types of chromosome preparation (*J. molec. Biol.*, **82**, 107; 1974). Both exhibit highly folded and tightly supercoiled DNA. The membrane-associated chromosomes show DNA fibres attached to one or two membrane patches and, since no free ends can be seen, the DNA is presumably intact in its usual circular state. The folded chromosomes seem to be less stable, more frequently generating breaks in DNA during preparation.

The topological problems posed by this compact structure of the chromosome remain formidable. Since strands of RNA can be seen in the folded structures, transcription must take place from sequences of DNA within the particle. Similarly, replication seems to proceed while the chromosome retains its compact structure. How DNA is able to unwind for these processes and how two daughter chromosomes may separate are among the most intriguing of the topological questions as indeed are problems concerning the access of enzymes and regulator proteins to particular sequences of DNA within the chromosome structure.

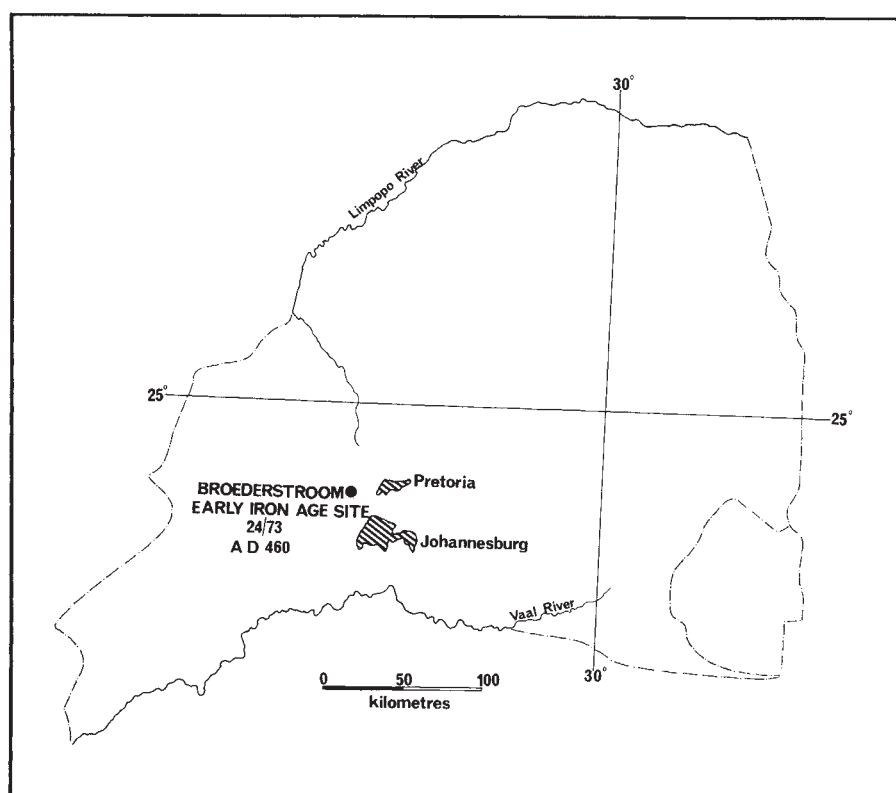
Early evidence of South African negroid

from a Correspondent

THE outline of an Iron Age village, which may represent the earliest and most intact village yet found in Africa south of the Sahara, has been discovered at Broederstroom near the Hartbeespoort Dam in Transvaal by a team led by Professor Revil Mason of the University of the Witwatersrand. The excavations followed up initial reports by Dr A. van Genderen who found the site. The material uncovered includes the earliest evidence of the South African negroid yet found and possibly also of domestic cattle.

The excavations at the site (no. 24/73) were conducted during the summer of 1973 in the grounds of the Leiden Observatory on the south bank of the dam and exposed thirteen huts and two iron slag floors on the perimeter and interior of an ellipse enclosing approximately 15 acres. No trace of stone walls was found suggesting that the village was enclosed by stockade. Among other material recovered were many thousands of sherds, a copper chain, disk ostrich shell beads, a cowrie shell, many hundreds of sandstone artefacts with 8–10 mm grooves (apparently for making shell beads), a few grindstones and some human remains.

Mason believes that the pottery was influenced by first millennium AD Iron Age pot makers in Zambia and in Malawi at Nkope and even as far north



as Kware in Kenya, and that it is also related to pots made in the coastal regions of Natal and as far south as Pondoland, where pottery similar to that in the 24/73 assemblage was found in the 1930s by J. F. Schofield. The single cowrie shell also suggests contact with the coast. The Broederstroom pottery form includes pots with everted rims, bowls with carinated profiles and curiously thickened rims, lugs and one spout. Decoration is broadline incision of triangular punctate only; stamping is absent.

The human remains suggest links with first millennium burial ritual at sites in northern Tanzania reported by Odner (*Azania*, **6**, 89; 1971.) Part of a maxilla of an adult human lying on the base of a clay pot next to part of a mandible of a 6–12 year old child, and parts of a human leg skeleton were recovered. These remains, according to Professor J. van Reenen of the University of the Witwatersrand School of Dentistry, fall within the range of present-day South African negroids, and, with a radiocarbon date of AD 460 $\pm$ 50 (obtained by R. Protsch at the University of California, Los Angeles), they are the earliest evidence of the South African negroid yet found.

Sheep or goat mandibles and molars of domestic cattle were also recovered and were identified by R. Welbourne. The cattle molars may be the earliest trace of domestic cattle yet found in South Africa, but their stratigraphy requires confirmation by the excavations planned for 1974. No plants were found during 1973, but it is possible

that flotation methods may reveal them during the next season.

Mason is confident that site 24/73 represents the earliest relatively complete settlement known to date in southern Africa, and that the pottery and burial methods indicate links with first millennium AD Iron Age cultures as far north as Kenya. The size of the slag floors, approximately 8 $\times$ 4 m each, and the quantity of grooved stones for bead making, suggest production in excess of local needs and may indicate trade links with neighbouring settlements not yet discovered. Mason has found similar pottery in Kruger Cave at Olifantsnek approximately 58 km west of the new site, and also near Thabazimbi 140 km north of 24/73. These records, together with those of other scattered Iron Age sites in Transvaal and Swaziland (see Mason, *Curr. Anthropol.*, **14**, 485; 1973), suggest that Iron Age settlement of South Africa during the first millennium AD was widespread. With the evidence of Broederstroom 24/73, archaeologists will now be able to relate these records to a village organisation of a kind associated with present-day Bantu speaking people in the rural regions of southern Africa.

How oil spills may affect the tundra

from our Plant Ecology Correspondent

THE exploitation of the oil fields in arctic North America has led to an increasing demand for information relat-

ing to its possible effects on the flora and fauna of the tundra biome. It is feared that the peculiar structure of tundra soils may render the system more sensitive to disturbance than has proved to be the case in more temperate climes. Below a depth of between 35 and 60 cm the soil is permanently frozen; the 'active layer' above this thaws and freezes according to the season. The application of physical pressures in the form of heavy vehicles is one form of disturbance which could upset this delicate system. Bellamy, Radforth and Radforth (*Nature*, **231**, 429; 1971) found that vegetation in wet areas regenerated well following compaction—ruts disappeared within 5 years—but ruts in dry areas were still distinct even after 20 years.

Damage to surface vegetation in tundra could have more than aesthetic significance; erosion of the superficial layers of peat could have economic repercussions if vehicular transport became difficult and communications were affected.

Attention has now turned to the outcome of oil spills in such terrain, for in time such an event must be inevitable. Not only is it liable to be toxic to the surface vegetation, but it could also influence the radiation balance of the system with possible effects on the permafrost. Wein and Bliss (*J. appl. Ecol.*, **10**, 671; 1973) have now described the outcome of experimental spills of crude oil in the Mackenzie Delta area of north-west Canada, 115 km from the Arctic coast. They chose different plant community types for their experiments, ranging from black spruce-alder communities on frost mounds to low lying sedge hollows in ice-wedge polygons. Plot sizes were between 3×4 m and 5×5 m depending on the community type, and oil was applied at various levels equivalent to 0.25 cm up to 1.50 cm. The second application is equivalent to 1,950 barrels per acre. Evaporation rates were estimated from open containers of oil, and temperature profiles in the peat together with the depth of the active layer were recorded.

Summer evaporation rates of oil were high, up to 15% volume losses being recorded within 16 h of application. Since many of the most phytotoxic components of crude oil are volatile aromatics, one suspects that this reduces the potential harm done by summer spills. On the other hand winter spills may not affect deep roots which are protected by permafrost. All lichens and all mosses except *Polytrichum juniperinum* were killed, and there was no recovery even a full growing season after application. The recovery of the remaining vegetation varied with species and with the level and season of applica-

tion; for example, *Carex* (sedge) species recovered less well following late season applications, whereas *Salix* (willow) species recovered better after autumn spills. In general it was the willows, birches and *Ledum palustre* (Labrador tea) which recovered most effectively from the oil applications. Total plant recovery was occasionally as great as 55% one growing season after the spill, which suggests that recovery of vegetation is best allowed to occur naturally, without further interference.

Temperature measurements showed that areas affected by spills absorbed more energy, so warming superficial layers. Increases in temperature, however, did not occur at great depth within the peat and the depth of the active layer was not affected. Probably much energy was lost in latent heat of evaporation and the drying surface peat would form an insulating cover over the cold, deeper layers.

It seems, then, that the tundra can probably cope with occasional, acute pollution from oil spills. It would be interesting to know whether this ecosystem can also survive low intensity, chronic pollution from the small spills which will inevitably be associated with the exploitation of these fields.

Half life of heavy chain message

from a Correspondent

IN estimating the turnover rate of mammalian messenger RNA there are two main problems—choice of suitable chase conditions and specific isolation of messenger RNA molecules for defined products. The presence of large pools of nucleotide precursors within eukaryotic cells creates obvious problems in a standard pulse-chase type of experiment. Decay of messenger in the presence of a metabolic inhibitor, for example actinomycin, is no longer considered to be satisfactory since the presence of drugs may cause generalised cellular malfunction so that synthesis and processing of RNA within such cells are abnormal.

Cowan and Milstein (*J. molec. Biol.*, **83**, 469; 1974) have approached the question with specific reference to the RNA species which codes for the heavy and light chains of an immunoglobulin secreted by a tissue culture-adapted mouse plasmacytoma. The cells were labelled with ^3H -uridine continuously during a period of 24 h in an automatic apparatus (see Cowan, and Milstein, *Biochem. J.*, **128**, 445; 1972), which allows the authors to calculate dilution due to growth, and thus correct the figure for RNA decay during the chase period.

Since no single chase procedure is entirely satisfactory several different conditions were studied. Cells were chased simply by the addition of cold uridine to the medium (the disadvantage of this procedure is continued and significant incorporation of low specific activity uridine during the chase which results in an overestimate of the messenger RNA half life). Alternatively, the cells were removed from the medium containing the isotopic uridine by centrifugation, and then resuspended in fresh medium containing 0, 0.1 or 0.2 M uridine. Exposure of the cells to uridine, however, causes appreciable swelling, and centrifugation is followed by a lag period of 3 h before re-entry into logarithmic growth. The messenger RNA molecules for heavy and light chains were purified by sucrose density gradient centrifugation of the microsomal extract, followed by selection of poly(A)-containing molecules using oligo(T)-cellulose. Purity of the heavy chain messenger was not known, and the light chain mRNA was 30% pure.

When radioactive uridine was removed for the chase period, similar half-lives for both heavy and light chain messenger preparations (12–14 h) were recorded whether cold uridine was present or absent. Thus, although none of these conditions is entirely satisfactory, the fact that the different chase conditions yielded similar results would suggest that the half-life values obtained were meaningful. Not unexpectedly the half-lives were longer (17–18 h) when the chase was done without removal of the radioactive uridine.

All of these values are much longer than those based on immunoglobulin synthesis in myeloma cells grown in the presence of actinomycin, probably indicating the unreliability of the actinomycin chase procedure. As the authors themselves clearly indicate, a crucial point is the purity of the messenger samples. Thus, values in the range 5–20 h cannot be excluded for the light chain message. Until the purity of the heavy chain message preparation is known, it would be premature to accept the half-life given as definitely that of heavy chain message. With increasing refinements in message RNA isolation and characterisation this question should be answered shortly.

Is Viking 1979 a possibility?

from our Cosmology Correspondent

THERE may be enough bits and pieces left over from the planned 1975 Viking missions to Mars to provide a low cost mission in 1979. Less probable, but still being actively considered, is a mission

including a Mars Rover, which could fly in 1979 or 1981 and would again use any spare parts from backups for the 1975 mission.

The 1975 missions will involve two landers and two orbiters, and a third lander is being built as a backup; this lander will, if all goes well, be available for a one-off mission in 1979, when an orbiter could be assembled from spare parts for the Viking 75 orbiters, according to a report in *Aviation Week and Space Technology* (February 11, page 56; 1974). This one-off mission would cost only some \$250 million to \$300 million, including the cost of a new scientific package. If a double mission like that of 1975 were to be attempted, one complete set of orbiter and lander would have to be built from scratch and this would push the total cost up to around \$500 million.

But perhaps the more intriguing possibility is that a Mars Rover vehicle could be added to the mission, at greater cost but without great technical difficulty. The estimated total cost of such a lander is some \$80 million and it is possible that the European Space Research Organisation (ESRO) would participate in this if the project goes ahead. Unfortunately, however, ESRO prefers the 1981 target date and this would involve NASA in much more expense, because of the need to keep Earth-based communications and staff available for another two years.

There is also the problem that NASA would like to do something in 1979 and if the 'spare parts' are used up then in a repeat of the 1975 missions, the cost of a 1981 rover mission would be increased by the need to build the lander and orbiter as well as the rover.

Leaving aside these difficulties for the present, just what would be involved for a lander-orbiter-rover mission in a few years time? The basic rover design suggests a 238-pound vehicle, which would add a total weight of 282 pounds to the Viking lander, including stowage and deployment mechanisms. The Martin Marietta Corporation sees no serious problems in having this vehicle ride piggyback on top of the Viking lander and suggests that this would only involve a slight loss of the lander's other scientific capability. As things now stand, the scientific payload of the rover, including camera, spectrometer, X-ray diffractometer and other experiments, would be 89 pounds.

The total weight of the lander-rover package would be 1,583 pounds; this is 106 pounds more than specifications for the 1975 landers define as safe, but it turns out that the rocket motors of these landers exceed their specification and that the maximum safe weight for the lander-rover could therefore go

up to 1,644 pounds if need be.

As well as making its own investigations over a range of 45 km, such a rover could make itself useful by off loading instrument packages from the lander and carrying them several metres away from the landing point, so that they could be deployed more efficiently.

Since the cost of integrating the rover into the lander system, which could be as much as 25% of the total cost of the rover, would be borne by NASA, it seems that ESRO could make a great contribution to the unmanned exploration of the planets for a cost of about \$60 million. This seems a small price to pay and it is to be hoped that ESRO is indeed taking the project seriously.

Amateur and professional entomology

from a Correspondent

THE entomological *Bulletin of the British Museum (Natural History)* is a handsome production, as befits so old and eminent an institution. Its recent issues, comprising volume 28 parts 6 and 7, and volume 29 parts 1 and 2, as well as supplement 20, provide good samples of the sort of systematic work that goes on within the museum walls. There are two works by members of the museum's staff, two by entomologists from other institutions who have worked on the museum's collections, and one by an amateur lepidopterist to show that an ancient and very English tradition is still upheld. One work provides a list of the numerous type specimens of an important butterfly genus held by the museum, two provide revisions of the species of particular genera, drawing more or less extensively on materials from other museums as well as the British Museum. The two largest parts are concerned with higher level groupings, an aspect of classification which sometimes tends to be neglected in publications of this kind. The two, however, differ profoundly in nature.

C. M. F. von Hayek, of the museum's staff, provides "A Reclassification of the Subfamily Agrypninae (Coleoptera; Elateridae)" (*Bull. Br. Mus. nat. Hist. (Entomology)*, Suppl. 20, 1-309; 1973) and the amateur J. N. Eliot deals with "The Higher Classification of the Lycaenidae (Lepidoptera)" (*ibid.*, 28, 373-508; 1973). Hayek's work is in the best professional tradition; she provides a very full discussion of the complicated synonymy in the group, a clear and workable key to genera and full synonymic catalogues of the species for each genus, with the designation of numerous lectotypes, many of them in foreign museums. She eschews any consideration of larval characters (known for many of

the species she deals with), habits or phylogeny, and is not very generous with illustrations, as compared with the other four authors. Her most questionable classificatory decision is the inclusion in Agrypninae of the genus *Octocryptus*, about which she herself expresses reservations.

Eliot provides no catalogues of species or even of genera, but provides keys down to the level of tribes and sections (= subtribes), and numerous illustrations of male genitalia and wing scales, including six plates of electron micrographs. A large part of his space is taken up with the systematic, adaptive and phylogenetic significances of characters of his butterflies, and he provides an extensive account of their phylogeny, including a dendrogram. The speculative nature of much of this is admitted by Eliot, and it remains for specialists in the group to evaluate his theories. He takes account of larval and pupal characters, geographical distribution and palaeogeography, but pays little attention to food plants (Ehrlich and Raven's celebrated *Butterflies and Plants: a Study in Co-evolution* is not even cited in his bibliography). Eliot's paper is designed to evoke thought and curiosity about his lycaenid butterflies, a result which Hayek is not likely to achieve for the Agrypninae, for all the usefulness her work will have for those concerned with the identification and arrangement of museum collections of the group. On the other hand, one feels that Eliot's work might gain from some injection of Hayek's formal procedural rigour, as she would certainly gain from his amateur breadth of outlook and intellectual curiosity.

New IR telescope

Buried in NASA's budget for 1975 is a little-noticed item which holds particular interest for astronomers. NASA is planning to build a large infrared telescope on Mauna Kea in Hawaii, some 14,000 feet above sea level. With a 3-m dish, it will be the largest infrared telescope in the world.

The chief reason for building the telescope—and the reason it is in NASA's budget rather than in that of the National Science Foundation—is to use it to get information on Jupiter, Saturn and their satellites in time for the 1977 Mariner Jupiter-Saturn flyby missions. Temperature profiles of the moons of those planets will be helpful in targeting the spacecraft to take a look at the most interesting features. Ultimately, however, the instrument, which will cost about \$6 million, will be used for general infrared studies of both galactic and extragalactic objects.

Infectious C-type virus isolated from a baboon placenta

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A C-type virus ("M7") has been isolated from a specimen of baboon placenta cocultivated with various mammalian host cell lines. Nucleic acid hybridisation with a ³H-DNA transcript of the viral genome demonstrates that this virus is genetically different from previously studied mammalian C-type viruses, and suggests that M7 is an endogenous baboon virus.

C-TYPE RNA-containing viruses have been isolated from tissues and cell lines of various mammalian species¹, including mice²⁻⁴, rats⁵, hamsters⁶, guinea pigs⁷, domestic cats⁸, pigs^{1,9}, woolly monkeys¹⁰ and gibbons¹¹. Because of the known aetiological role of these viruses in the generation of vertebrate neoplasms¹², there have been extensive attempts to isolate infectious C-type viruses from other primates, including man. Recent electron microscopic evidence indicates that C-type viruses are present in the placentas of baboons¹³ and rhesus monkeys¹⁴, and, less frequently, human trophoblastic tissue¹⁵. We describe here the isolation and *in vitro* propagation of a typical C-type virus from a normal baboon placenta. Nucleic acid studies suggest an endogenous baboon origin for this virus isolate and demonstrate that it is distinct from known described mammalian C-type viruses, including two of presumed primate origin.

First trimester placental tissue was obtained by Caesarian section from an 11-yr-old multiparous baboon (*Papio cynocephalus*) from the colony maintained at the Southwest Foundation for Research and Education, San Antonio, Texas. This animal was imported to the United States from Africa in 1968 and had not been used previously for medical research. A portion of the placenta was removed for viro-

logical studies and the remainder was processed for nucleic acid extraction. Small pieces of the placenta were cocultivated directly with the indicator cell lines described in Table 1. In addition, a 5 g sample was homogenised in a Teflon pestle tissue grinder, suspended in 15 ml of cell culture medium, and centrifuged at 1,000*g* for 20 min. The supernatant was filtered through a 0.45 µm Millipore filter, and used to infect various mammalian cell lines, some of which are highly permissive for endogenous mouse and cat C-type viruses^{16,17}. The cultures were transferred every 2 weeks, and, in most cases, by the third transfer there was no evidence of baboon placental cells remaining in the cocultivated cultures.

Cultures were tested for C-type virus production by assaying for viral reverse transcriptase in the tissue culture media¹ (Table 1). After 5 weeks of cocultivation, C-type viral replication was first detected in FCf2Th, a foetal canine cell line. By 13 weeks, viral replication could also be detected in the rhesus monkey cell line, DBS-FRhl-1, in the bat lung cell line, Tb1Lu, and in the human rhabdomyosarcoma line, A204. Viral replication was also detected after 13 weeks in the cultures of A204, DBS-FRhl-1 and Tb1Lu which received filtered extracts of the baboon tissue (Table 1). The four other cell lines remained virus-negative during the 3-month test period.

The polymerase-positive foetal canine cell culture, FCf2Th, was examined by electron microscopy. As Fig. 1 shows, typical C-type viral particles were present in the extracellular space and in intracytoplasmic vacuoles, and were seen budding from the cell membranes. Designated M7, this virus rapidly generated productive infections of previously virus-free cultures of A204, DBS-FRhl-1, Tb1Lu, the mink cell line Mv1Lu, the dog cell line MDCK and the equine cell line E. Derm. No viral replication was detectable in rat NRK cells or in a foetal cat strain, FFe60WF,

TABLE 1 Growth of Baboon Placental Virus on Various Mammalian Cell Lines

Cell line (or strain)	Origin	Days after cocultivation					Supernatant reverse transcriptase assay (c.p.m. × 10 ⁻³ , ³ H-TMP incorporated)	
		15	23	36	60	90	Days after infection with filtered extract	
A204	Human rhabdomyosarcoma ³¹	0.9	3.5	10.0	20.7	749.8	60	810.0
A1215	Gibbon connective tissue	0.4	0.7	1.4	NT	1.8	90	NT
DBS-FRhl-1	Foetal rhesus lung ³²	2.6	3.2	1.7	33.8	1025.6	60	1080.2
FCf2Th	Foetal canine thymus (NBRL)	1.1	1.0	209.6	227.7	NT	60	NT
SIRC	Rabbit cornea (ATCC)	0.6	0.6	2.7	5.6	2.9	90	5.3
Tb1Lu	Bat lung (ATCC)	0.8	0.6	1.5	4.5	762.6	60	337.0
FFe60WF	Foetal cat (NBRL)	1.6	1.5	1.5	3.3	2.3	60	NT
Bu(IMR-31)	Buffalo lung (ATCC)	1.0	2.1	2.1	2.4	1.5	90	2.5

Cell culture medium was assayed at various times after cocultivation or infection for reverse transcriptase activity as previously described¹. Results are expressed as c.p.m. of ³H-TMP incorporated into poly(dT) product during a 60 min incubation at 37°C (40,000 c.p.m. minute is equivalent to 1 pmol of ³H-TMP). ATCC, American Type Culture Collection, Rockville, Maryland; NBRL, Naval Biomedical Research Laboratory, Oakland, California.

NT, not tested.

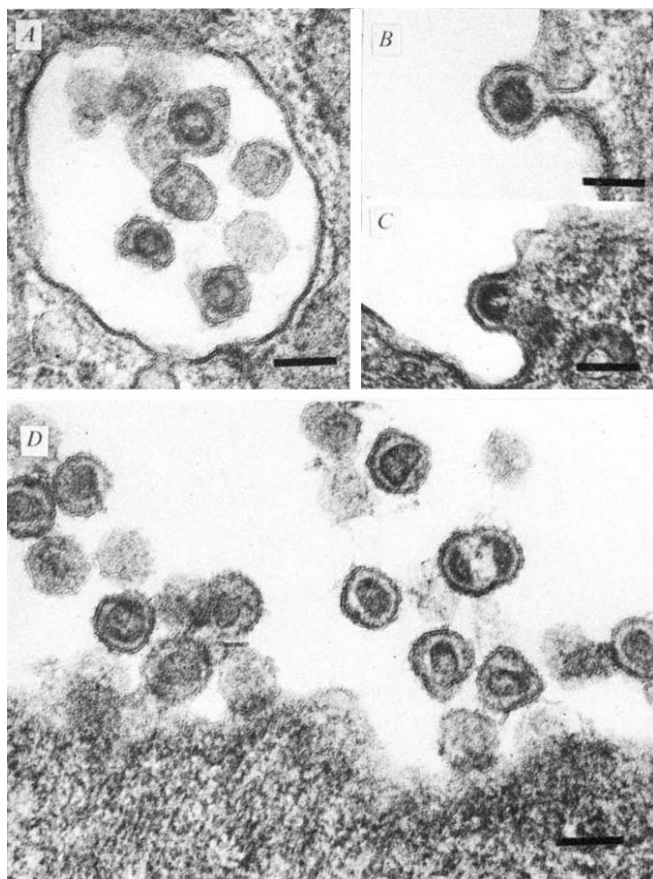


Fig. 1 Electron micrographs of C-type virions released by a foetal canine thymus cell line (FCf2Th) infected with an extract from a baboon placenta. Cell monolayers were fixed with buffered 2% glutaraldehyde and then scraped off with a rubber policeman. Cells were then sedimented and fixed in 1% buffered osmium tetroxide. After overnight incubation in cold 0.5% uranyl acetate, the cells were dehydrated and embedded in Epon. Thin sections were doubly stained in uranyl acetate and lead citrate and examined with a Siemens Elmiskop 1A electron microscope. A, intracellular particles. B and C, particles budding from the foetal canine cells. D, extracellular C-type particles. The scale represents 100 nm.

that is permissive for both feline leukaemia virus and endogenous feline C-type viruses (RD114/CCC group)¹⁶.

M7 virus was grown in FCf2Th cells, concentrated by continuous flow centrifugation, and further purified by isopycnic banding on sucrose gradients. The peak of reverse transcriptase activity occurs at a density of 1.16 g cm^{-3} , characteristic of C-type viruses. Banded virus was disrupted with detergent and chromatographed on a Sephadex G-100 column calibrated with several protein markers. The reverse transcriptase activity eluted at a position corresponding to a molecular weight of 70,000, similar to that reported for other mammalian C-type viral polymerases¹⁸. Enzymatic activity assayed with a poly(A) template and oligo (dT)₁₂₋₁₈ primer showed the divalent cation preference for Mn^{2+} over Mg^{2+} , characteristic of mammalian C-type viruses¹⁹.

The relatedness of the M7 virus to other C-type viruses was determined by comparing nucleic acid homology using DNA-RNA hybridisation. A single-stranded ^3H -DNA copy of M7 viral RNA prepared using endogenous RNA-dependent DNA polymerase in the presence of actinomycin D²⁰ was annealed to viral RNAs or to cytoplasmic RNA extracted from cell lines that release C-type virus. Table 2 shows representative data obtained after digestion of the RNA-DNA hybrids with the single-strand specific nuclease,

S_1 ²⁰⁻²². The data have been normalised with respect to the final percentage saturation hybridisation values obtained with M7 viral RNA; these values ranged from 50-60%. Interpretation of RNA-DNA hybridisation using ^3H -DNA prepared from an endogenous reverse transcriptase reaction is limited since the probe need not represent equally the entire viral RNA²³. Thus, homologies between infrequently transcribed portions of the M7 genome and other viral genomes may not be detected. Nevertheless, the data show that viral RNA extracted from three murine viruses, a rat virus, a feline virus, a Chinese hamster virus, a pig virus, and two presumed primate viruses (woolly monkey and gibbon) have less than 2.0% homology to the ^3H -DNA probe prepared from M7. The probe hybridised to a small extent (10%) with an endogenous feline virus, RD114; the significance of this relationship is not yet clear. These data

TABLE 2 Nucleic Acid Homology of the M7 ^3H -DNA Product to other C-type Viral RNAs

C-type virus	Origin	Percent M7 ^3H -DNA hybridised
M7	Baboon placenta	100.0
Gibbon	Gibbon lymphosarcoma	1.5
Woolly	Woolly monkey sarcoma	2.0
Feline	Cat leukaemia (Rickard)	1.8
	Human rhabdomyosarcoma cell line passaged through kittens (RD114)	10.0
Murine	Mouse leukaemia (Rauscher)	0.0
	Transformed mouse cell line (S16CL2)	0.5
	Human rhabdomyosarcoma cell line passaged through NIH Swiss mice (AT-124)	0.0
Chinese hamster	Hamster peritoneal cell line (B14-I50)	1.4
Rat	Rat thymus cell line (RT21c)	2.0
Porcine	Pig kidney cell line (PK-15)	0.0

Approximately 2000 c.p.m. (0.07 pmol of ^3H -TMP incorporated into DNA) of a single-stranded M7 ^3H -DNA product was hybridised to either viral or cytoplasmic RNA²⁰. The data shown represent the averages of final saturating percentage values obtained after adding increasing amounts of viral RNA (up to 20 μg) or cytoplasmic RNA (up to 2 mg) extracted from a virus-producing culture. The percentage hybridisation values have been normalised with respect to the final percentage hybridisation obtained with the homologous M7/FCf2Th RNA (actual values ranged from 50-60%). Hybridisation was carried out for 72 h at 41°C in the presence of 38% formamide as previously described²⁰. The formation of ^3H -DNA-RNA hybrids was detected with S_1 nuclease²⁰⁻²². RNA extractions were performed as described previously^{20,23}. S16CL2, PK-15, B14-I50 and RT21c are endogenous viruses spontaneously released from their respective mouse, pig, Chinese hamster and rat cell lines. The Rickard strain of feline leukaemia virus was grown in a cat thymus cell line (F422), Rauscher murine leukaemia virus in the murine line JLSV-9, RD114 and AT-124 in a human rhabdomyosarcoma cell line (RD), gibbon and woolly monkey leukaemia viruses in the human rhabdomyosarcoma cell line (A204), and M7 in the foetal canine thymus cell line, FCf2Th. These viruses and their host cell lines have been described previously²³. Viral 70S RNA was extracted²³ from gibbon, woolly monkey, feline and murine viruses. The source of rat, porcine, Chinese hamster and M7 viral RNA was cytoplasmic RNA extracted from cultures producing these viruses. Both sources of RNA have been shown to yield similar data²³.

are also obtained when a reverse transcript prepared from each of the viruses listed in Table 2 is annealed to M7 viral RNA. Immunological studies of M7 viral reverse transcriptase and group-specific protein also demonstrate that the virus can be distinguished from known C-type virus isolates. These results argue against the possibility that the M7 isolate resulted from inadvertent infection of the animals, specimens or cell cultures by known laboratory C-type viruses.

To ascertain whether M7 could be an endogenous baboon virus, a single-stranded ^3H -DNA copy of M7 RNA was

annealed to DNA extracted from a normal baboon liver. As Table 3 shows, the M7 probe hybridised fully to the DNA extracted from baboon liver and from the placenta from which the virus was isolated. Thus, sequences in the DNA of the liver and placenta from two baboons are completely homologous to those found in the M7 virus that was grown in dog cells. Tissues from four other baboons also contain DNA homologous to the M7 viral DNA probe (unpublished experiments). In contrast, DNA extracted from dog and pig liver, from a murine cell culture producing mouse C-type virus (S2CL3), and from the uninfected foetal canine cell line FCf2Th hybridised to less than 6% of the probe. There is a small amount of M7-related information in feline liver DNA (15%); the nature of this feline-baboon viral homology is examined in a separate report.

Cytoplasmic RNA extracted from the normal baboon liver and from the baboon placenta from which M7 was isolated was tested for its ability to anneal to the M7 ³H-DNA probe. Table 4 shows that whereas M7-specific viral RNA is readily detectable in baboon placenta, the addition of up to 0.9 mg of RNA from the baboon liver resulted in less than 2.6% hybridisation to the M7 probe. Thus, M7 viral information, though present in the cellular DNA, is not extensively transcribed in normal baboon liver tissue.

C-type viruses have been observed by electron microscopy in baboon placental tissue, and the hybridisation data demonstrate that RNA homologous to the M7 virus genome is being produced. In contrast, viruses have not been seen in adult baboon liver, and M7 viral RNA is not detectable by nucleic acid hybridisation. Thus, while viral genetic information is present in the DNA of both a virus-producing tissue (the placenta) and an apparently virus-free tissue (the liver) only the placental tissue is 'switched on' for virus production. The physiological basis for control of C-type virus expression and the mechanisms that allow virus activation in placental tissue remain to be elucidated.

These findings suggest that M7 is an endogenous C-type virus of the baboon. Similar nucleic acid hybridisation experiments have previously shown that chickens²⁴⁻²⁶, cats²⁷⁻²⁹ and pigs³⁰ also have vertically transmitted C-type viral genomes. Since ³H-DNA probes prepared from the woolly monkey virus and the gibbon ape virus show little or no homology to the DNA extracted from normal woolly or gibbon tissues (Scolnick *et al.*, manuscript submitted; Ben-

cocultivation of suspected virus-producing tissues with cells from various species to maximise the probability of finding a permissive host. The same general approach may be applicable in attempts to isolate infectious C-type viruses from primates other than the baboon and from tissues other than the placenta.

TABLE 4 Expression of M7 Virus-specific RNA in Baboon Liver and Placental Tissue

Tissue	µg RNA added	Percentage M7 ³ H-DNA hybridised
Placenta	94	12.0
	282	22.5
	940	40.5
Liver	90	1.8
	450	2.6
	900	2.4

Approximately 1,600 c.p.m. of a single-stranded M7 ³H-DNA probe was hybridised to increasing concentrations of cytoplasmic RNA extracted from the baboon placenta from which M7 was isolated and from a normal liver of another baboon. The percentage hybridisation values have been normalised with respect to the final percentage hybridisation obtained with RNA extracted from the M7-infected foetal canine cell culture, FCf2Th; this value ranged from 50-60%. See legend to Table 2.

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TABLE 3 Hybridisation of M7 ³H-DNA to DNA extracted from Tissues of Various Species

Species	Tissue	Percentage M7 ³ H-DNA hybridised
Baboon	Placenta	100.0
	Liver	115.0
Canine	Liver	5.0
	Cell culture (FCf2Th)	6.0
	Cell culture (FCf2Th) (M7 infected)	100.0
Feline	Liver	15.0
Porcine	Liver	1.0
Murine	Cell culture (S2CL3)	2.0

The data show normalised percentage hybridisation values to the M7 ³H-DNA probe obtained after annealing to cell DNA to a *Cot* value of 8×10^3 (corrected for salt concentration to 0.12 M phosphate as described by Britten and Smith³⁴). DNA-DNA hybridisations were performed as previously described³⁰; the hybrids were detected with S₁ nuclease. DNA extractions were performed as described previously³⁰. The ratio of cell DNA (300 µg) to ³H-M7 DNA (0.1 ng) was 3×10^6 .

veniste *et al.*, manuscript submitted), M7 seems to be the first example of a vertically transmitted C-type virus of primates.

Our experiments demonstrate the validity of a general strategy for *in vitro* isolation of C-type viruses involving

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Three-dimensional structure of yeast phenylalanine transfer RNA at 3.0 Å resolution

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At 3 Å resolution, the electron density map of crystalline tRNA shows the polynucleotide chain as an alternating series of ribose and phosphate peaks. Bases are seen, especially in the double helical stem regions. A complete three-dimensional model of the L-shaped molecule has been built.

TRANSFER RNA is the molecule on which the polypeptide chain is assembled when tRNA interacts with mRNA. There is considerable interest in the three-dimensional structure of this molecule as a first step in understanding the detailed mechanism of protein synthesis. For several years we have been analysing crystals of yeast phenylalanine tRNA. The molecule crystallises in an orthorhombic unit cell ($P2_12_12_1$, $a = 33\text{Å}$, $b = 56\text{Å}$, $c = 161\text{Å}$) and these crystals produce high resolution X-ray diffraction patterns¹. We obtained heavy atom derivatives which made it possible to calculate an electron density map initially at a resolution of 5.5 Å² and at 4.0 Å resolution³. From the latter study it was possible to discern the overall shape of the molecule and show that the molecule was L-shaped with one arm of the L containing the CCA and TΨC stems while the other arm contained the dihydrouracil (hU) and anticodon stems which are components of the familiar cloverleaf arrangement of the polynucleotide sequence of yeast phenylalanine tRNA⁴ (Fig. 1). At 4 Å resolution the ribose-phosphate groups could be seen as individual peaks and the tracing of the chain was carried out largely by following adjacent peaks in the electron density map. Now we report that at 3 Å resolution the phosphate groups are typically seen as individual peaks separated from the ribose peaks. In addition, many of the purine and pyrimidine bases can be seen, especially in those regions in which base pairs are formed. The tracing of the polynucleotide chain seen at 3 Å resolution is virtually the same as that seen at 4 Å resolution, but many more details begin to emerge.

Structure and electron density

The experimental details for crystal growing and heavy atom derivative formation as well as data collection have been described previously^{1,2}. We have explored more fully

the conditions under which heavy atom derivatives can be formed and have developed several more heavy atoms sites, whose positions in the unit cell are listed in Table 1. The most important of these are the samarium and osmium derivatives. There are four samarium sites, two of which have major occupancies and two of which are less fully occupied. The osmium derivatives have in addition to the major site described previously², two sites with lesser occupancies. A double derivative has been obtained by soaking crystals initially with potassium osmate and then with samarium acetate. In addition, we have platinum de-

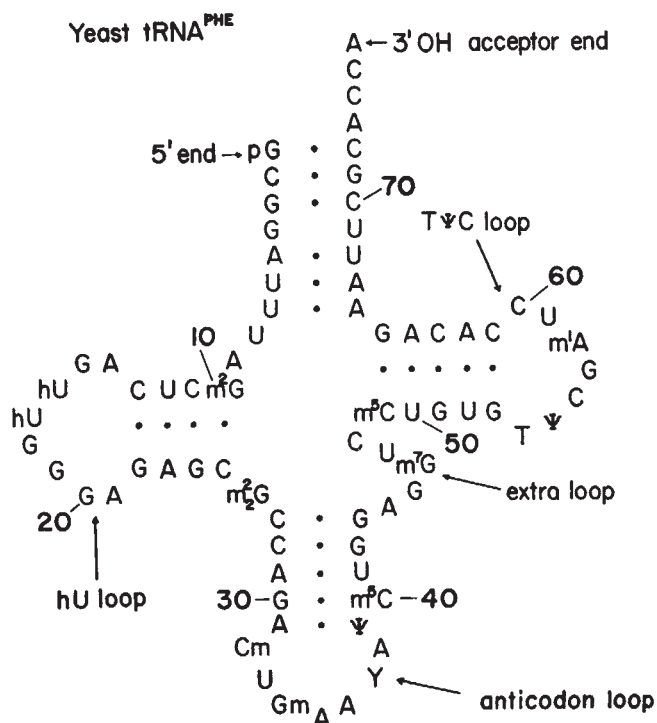


FIG. 1 The nucleotide sequence of yeast phenylalanine tRNA in the conventional cloverleaf diagram⁴. The nucleotides are numbered starting from the 5' end.

rivatives containing four sites and praseodymium derivatives with the same four sites as those found in the samarium derivatives.

The 3 Å sphere contains 6,807 unique reflections for this unit cell and of these, 4,902 were used in calculating the three-dimensional electron density map. Bijvoet pair data was collected for the derivatives. The overall figure of merit is 0.66 for the data used and the various residuals for each of the different derivatives are listed in Table 2. It can be seen that the most useful data was that produced by the samarium and osmium-samarium derivatives. The platinum data had only limited value and the osmium data was useful likewise only at low resolutions. A full crystallographic report will be published elsewhere.

Three-dimensional electron density maps were plotted at intervals of approximately 0.8 Å along the *a*, *b* and *c* axes. The map sections perpendicular to the *b* axis were used in assembling molecular models because of the more ready visualisation of the molecule. The electron density contours were drawn on glass or plastic sheets at a scale of 2 cm Å⁻¹ which were then mounted in a Richard's box⁵.

TABLE 1 Heavy atom parameters

	X	Y	Z	Occupancy*
Sm	0.555	0.169	0.275	M
(3.0 Å)	0.279	0.373	0.232	M
	0.849	0.105	0.215	I
	0.188	0.175	0.157	W
Os-Sm	0.558	0.167	0.275	I
(3.0 Å)	0.282	0.372	0.232	M
	0.867	0.106	0.214	I
	0.198	0.179	0.160	W
	0.035	0.196	0.065	I
Os	0.029	0.193	0.066	M
(4.0 Å)	0.006	0.274	0.231	I
	0.073	0.234	0.051	I
Pt	0.040	0.189	0.061	I
(4.0 Å)	0.198	0.148	0.036	I
	0.105	0.224	0.324	I
	0.244	0.417	0.114	I
Pr	0.571	0.168	0.280	I
(5.0-4.0 Å)	0.277	0.376	0.233	I
	0.829	0.101	0.215	I
	0.187	0.172	0.158	I

* Occupancy: M, major site; I, intermediate site, W, weak site.

between the 3' hydroxyl group at one end of the L-shaped molecule and the anticodon bases at the other end of the L is 77 Å.

TABLE 2 Refinement statistics

	Sm	Os-Sm	Os	Pt	Pr
N	4843	3309	2773	1943	556
Resolution(Å)	3.0	3.0	4.0	4.0	5.0-4.0
R_K	0.094	0.110	0.194	0.310	0.087
R_M	0.63	0.73	1.18	2.52	0.74
R_W	0.49	0.68	1.53	7.76	0.72

N, number of reflections; the residual values R_K , R_M and R_W are defined in ref. 2.

Chain tracing

In attempting to trace the chain from the electron density map, several criteria and constraints were used. The first constraint is the fact that the polynucleotide backbone can be extended to a maximum repeat distance of approximately 7.5 Å or a minimum distance of approximately 4.5 Å. Second, the chain needs connectivity, that is, it should be traceable over its entire length without any gaps in the electron density map. Third, there must be a relationship between the total electron density in the map and the length of polynucleotide chain which is used to interpret the map. All or most of the high density regions in the map should be accounted for. Fourth, the polarity of the polynucleotide chain can be determined in double helical regions. This is due to the fact that these regions show both major and minor grooves in the electron density map. In interpreting these maps, we used conformational data from the fibre diffraction analysis of double helical RNA⁷ as well as from the recent single crystal analyses of fragments of RNA double helix^{8,9}. These studies provided models for interpreting the right-handed double helical stem segments and they unambiguously fix the direction of the polynucleotide chain.

In regions of the map which do not contain double helical segments, however, the connectivity and polarity of the chain is more difficult to determine. In these regions we try to recognise the base peaks and utilize conformational information obtained from the single crystal structure analysis of oligonucleotides⁸⁻¹¹. In addition, we have used the secondary structure implicit in the cloverleaf formation shown in Fig. 1

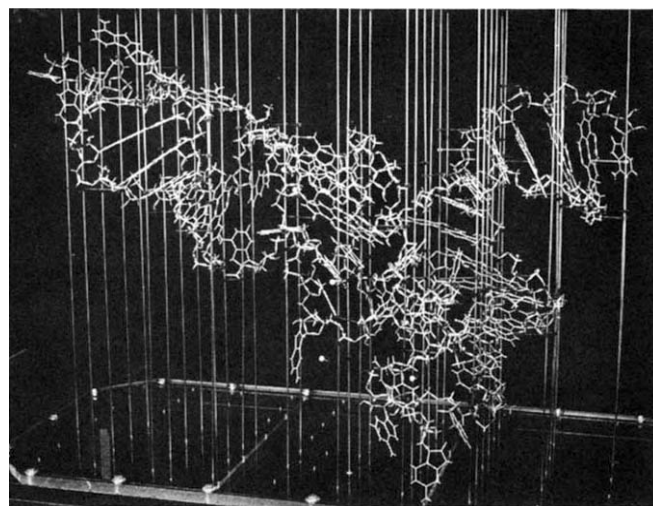


FIG. 2 A photograph of a Kendrew wire model of yeast phenylalanine tRNA based on the 3 Å resolution electron density map. The model is built at a scale of 2 cm Å⁻¹. The CCA stem is at the upper right. The TΨC and hU loops are at the bottom and the anticodon loop is at the upper left. The L shape of the molecule is evident.

Kendrew wire models were used to build the molecule. The electron density map showed the gross outlines which were described previously in the 4 Å map. In addition, the 3 Å map revealed much more detail than was available at 4 Å. Substantial regions of the map are occupied primarily by solvent and there is very little accumulation of electron density in these areas. A channel of approximately 36 × 40 Å runs parallel to the *a* axis throughout the crystal and it permits the cell shrinking phenomena described earlier⁶. The double helical stems which were recognisable at 4 Å resolution now become clearly resolved; ribose and phosphate groups can be readily discerned from each other over most of the stem regions and the characteristic zigzag array of these two groups provide the continuity needed for tracing the backbone of the molecular chain. Figure 2 shows a photograph of a Kendrew wire molecular model built to fit the electron density in the map. In this view the anticodon is in the upper left and the CCA stem is in the upper right with the TΨC and hU loops forming the corner at the bottom. The L-shaped form of the molecule is readily apparent. The entire asymmetric unit is shown in this view with the exception of three terminal nucleotides CCA at the 3'OH end. The CCA end of one molecule extends into the neighbouring unit cell where it rests close to the anticodon stem of an adjacent molecule. It was not included in Fig. 2 to increase the clarity of the photograph. The distance

as an adjunct for the chain tracing. Although the map contains double helical stem regions in which the chain tracing is unambiguous, there are areas in which the chain tracing is less clear. In particular, the T Ψ C and hU loop areas are close together near the corner of the L and both chains follow a fairly irregular course. Furthermore, there are additional peaks which we interpret as tightly bound cations. This makes the chain tracing in these areas much less certain than in the double helical stem regions.

As mentioned above the three nucleotides CCA at the 3'OH end of the molecule extend out from the CCA stem and are adjacent to the anticodon stem of a neighbouring molecule. The electron density peaks are slightly lower in the CCA region of the molecule than elsewhere, suggesting somewhat greater disorder in that part of the molecule. The 3' terminal ribose peak is found 3 Å from the major osmium site, in agreement with our suggestion that this site may be due to the formation of an osmium-bipyridine complex with the two *cis* hydroxyl groups of the 3' ribose³. But as we have found two additional osmium sites, it is clear that this is not the sole mechanism by which osmium can complex to RNA molecules. In this regard it is likely that the osmium derivative reported by Schevitz *et al.*¹² represents an alternative mode of complexing.

The three-dimensional Kendrew model was built first by assembling the double helical segments while paying attention to the geometry of these segments as discussed above. A very long double helical segment containing approximately one turn of the helix is seen at one end of the molecule (right side of Fig. 2), the 3' end of which is attached to the isolated segment of four nucleotides which we interpret as the ACCA end. Once the double helical stem segments were built, the identification of loops was guided by the clover-leaf diagram. In general, we are reasonably confident about the assignment of residues in the double helical stem segments. We are not as confident of the loop areas, however, since there are often ambiguities regarding continuity where there are many close contacts.

Figures 3, 4 and 5 show three sections of the electron density map perpendicular to the *a*, *b* and *c* axes. These maps are presented to illustrate the kind of resolution seen in the electron density map, and at the same time the superimposed heavy black lines illustrate our interpretation of those regions of the map. Figure 3 shows a slice, perpendicular to the *c* axis, which passes through one strand of the anticodon stem. It can be seen that there is a reasonable resolution of ribose and phosphate peaks and two of the base pairs in the stem region lie almost in the plane of this slice. Although the base pair can be seen in the map, it is clear that we cannot differentiate one base from another at this resolution. There are sections of electron density in the upper right and lower right of Fig. 3 which arise from neighbouring molecules.

A section perpendicular to the *b* axis is shown in Fig. 4. The large empty area to the left is the region filled largely by solvent and it is this region which disappears when the crystals are transformed into a smaller unit cell by shrinkage⁶. This section contains an elongated length of polynucleotide chain including part of the hU stem region. The zigzag nature of the ribose phosphate chain can be seen fairly clearly. The vertical *a* direction contains one and a half unit cells because the molecule itself spans more than one unit cell in the *a* direction. All the labelled residues in Fig. 4 are in one molecule. The unlabelled parts are related to the labelled parts by symmetry, either a translation in the vertical *a* direction or a two-fold rotation as indicated by the axes shown in the figure. The peak labelled M is interpreted as a metal ion which is found in the position where two molecules are in close contact.

Figure 5 shows a section perpendicular to the *a* axis. The

arc of electron density seen at the left of the figure is a portion of the T Ψ C stem which lies in the *bc* plane. The section at the far right cuts through the anticodon part of the molecule one unit cell below whereas the portion in the middle which contains very little electron density lies between two molecules.

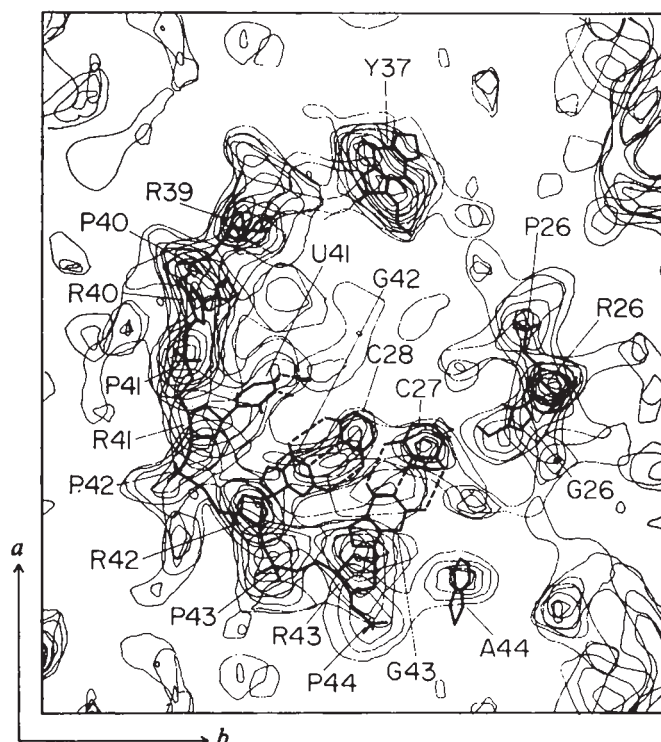


FIG. 3 A composite of electron density map sections cut perpendicular to the *c* axis in the interval $z = 0.110$ – 0.120 . The vertical distance is slightly greater than an *a* axis repeat of 33 Å. The horizontal axis is close to one half of the 56 Å *b* axis. The heavy lines represent portions of the molecule, and a part of the anticodon stem is seen. The electron density at the upper right and lower right arise from adjacent molecules in the next unit cell. P, phosphate group; R, ribose.

As is obvious from Figs 3, 4 and 5 most of the peaks of electron density are accounted for by structural features of the molecule. Some peaks, however, are not accounted for by the polynucleotide backbone. Many of these are believed to be ions, especially since a number of them are found within 4 Å of the negatively charged phosphate groups. In addition to the magnesium ions, the solvent also has the arsenic-containing cacodylate ions of the buffer as well as the polycationic spermine. Some additional experiments are necessary in order to ascertain the nature of these additional peaks in the electron density map.

Molecular structure

There are a few important features of the connections between the stem areas that should be described. In going from the CCA stem to the hU stem there are two nucleotides, 8 and 9, where the chain makes an abrupt change in direction. The uracil in position 8 was found to be partially stacking over the cytosine of nucleotide 13; thus the photodimerisation reported by Yaniv *et al.*¹³ can easily be explained by this juxtaposition. We have made a reasonable tracing of the hU loop in which the guanine of nucleotide 15 forms a hydrogen bonded pair with the cytosine of nucleotide 48. This base pair has been described as a possible feature of the secondary structure of tRNA¹⁴. However, since this occurs in a loop region, we must still regard this as somewhat tentative.

Previously we described the TVC stem and the CCA stem as organized around one helical axis while the anticodon stem and the hU stem were located around another helical axis approximately at right angles to the first³. At higher resolution the CCA and TVC stems are still seen organized around approximately the same helical axis. The hU stem is organised around an axis which is not co-linear with the anticodon stem however, but diverges from it by an angle which may be as great as 30°. It is somewhat difficult to make this measurement since it is not easy to define the helical axis of a short, somewhat irregular helical stem. The angular divergence is, however, great enough so that attempts to locate the double helical segments by crystallographic search techniques are not likely to be successful. We have looked at the helices found in the various stems and we find that they are not completely regular so that these helices cannot be described in simple terms as belonging to one or another of the classes of standard RNA helices⁷. This finding is not unreasonable in view of the fact that the double helical stem regions are short and have many other interactions in addition to those which determine the geometry of an extended double helical molecule. These interactions are undoubtedly reflected in modifications of the helical parameters.

The electron density in the CCA stem shows only a slight irregularity in the region of the guanine-uracil base pair; thus we can say that these bases are not folded out of the stem, but rather are found stacked within the double helical portion of the molecule. Further work, however, is needed to show whether or not these bases are hydrogen bonded in the stem.

The anticodon loop can be described generally as one in which the ribose and phosphate groups are on the inside and the bases are on the outside. The geometry of the loop does not agree with earlier proposals that the helical conformation continues up to the anticodon on either the 3' or 5' side^{15,16}. It is possible, however, that the conformation of the anticodon loop in solution may be different from that in the crystal. One interesting feature in the map is the very large Y base at position 37 which is found on the 3' side of the anticodon loop¹⁷. It is seen in a position such that its elongated side chain extends across the major groove of the anticodon double helix so that its end is adjacent to a phosphate group on the hU stem. The final details of the position of this base as well as the anticodon bases must await further refinement and data collection.

The diffraction pattern of this orthorhombic unit cell extends out to a resolution of 2.3Å, thus almost twice as

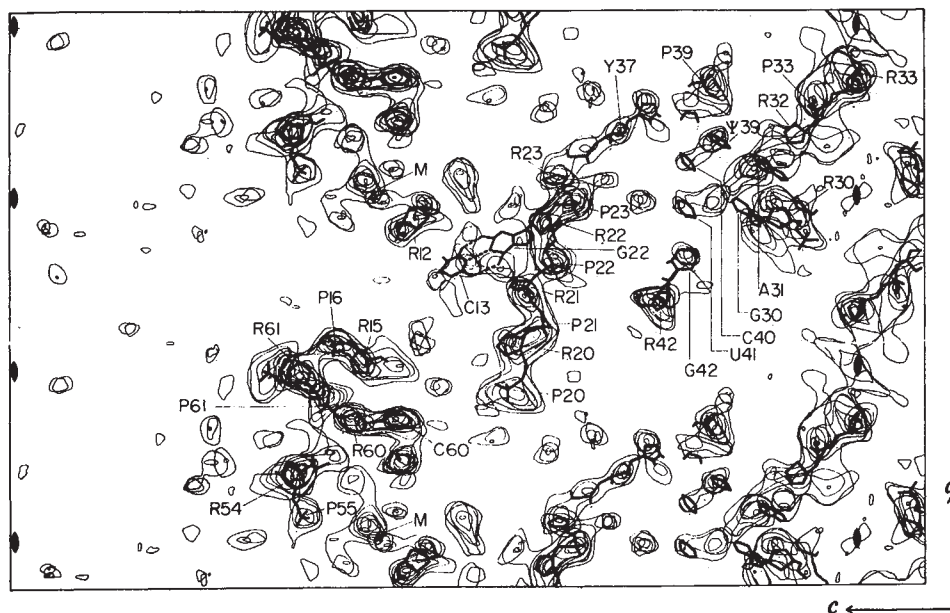


FIG. 4 A composite of electron density map sections cut perpendicular to the *b* axis in the interval $y = 0.125-0.165$. The two-fold rotation axes are drawn in at the right and at the left, defining the horizontal *c* distance of one half of 161 Å. One and a half unit cells are shown in the vertical *a* direction in order to include one molecule. The labelled molecular units are all part of one molecule; the unlabelled portions belong to symmetry related molecules.

Another feature of the model is the fact that the single purine residues which are found at the end of double helical stems often lie in a stacked configuration on the stem. This is particularly true of the adenine residue in position 73 at the end of the CCA stem, the dimethylguanine in position 26 which stacks on the anticodon stem as well as the adenine in position 14 which stacks on the end of the hU stem.

much diffraction data can be collected by extending the study from 3Å to 2.3Å. We are continuing the analysis in this direction and are also continuing the search for additional heavy atom derivatives which will help to define further the phases used in calculating the three-dimensional electron density map. At present we are confident of the overall form of the molecule and can define in reasonable detail the

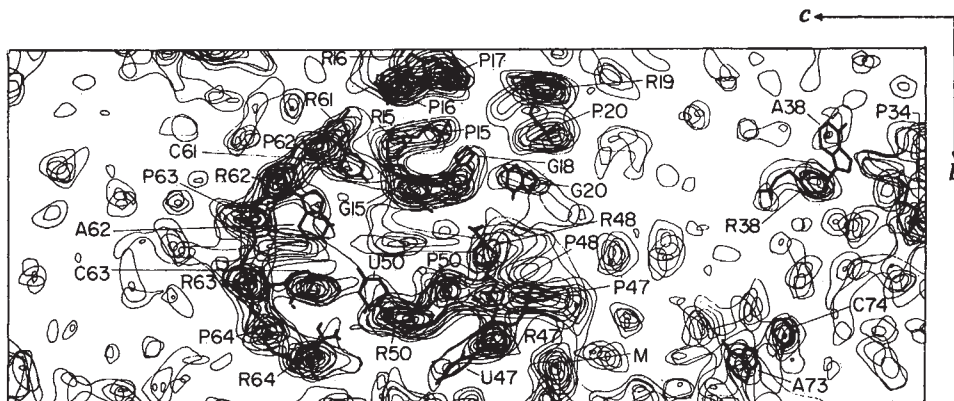


FIG. 5 A composite of electron density map sections cut perpendicular to the *a* axis in the interval $x = -0.05$ to 0.025 . The horizontal axis includes one half the *c* axis; the vertical axis has one half the *b* axis. The residues labelled A73 and C74 belong to a molecule in the adjoining unit cell. An arcing part of the TVC stem is seen.

double helical stem regions. The loop areas, however, remain somewhat uncertain and although we can make quite reasonable chain tracings we cannot be certain that these chain tracings are unique. This is of particular importance in that we would like to define completely the conformation of the hU loop as well as the detailed and interesting interaction between the T Ψ C and hU loop areas in the corner of the molecule. We hope that these will emerge in our continuing study.

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Morphine-like drugs inhibit the stimulation by E prostaglandins of cyclic AMP formation by rat brain homogenate

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In homogenate of rat brain, morphine, at concentrations obtainable in vivo, inhibited the stimulation by prostaglandin E₁ or E₂ of cyclic AMP formation, without inhibiting the basal production of cyclic AMP. Heroin was more and methadone less active than morphine, whereas dextrorphan was inactive and naloxone antagonised the effect of morphine. This inhibition may represent a mechanism whereby morphine-like drugs exert their analgesic or other effects.

EXPERIMENTAL administration of E prostaglandins elicits the main pharmacological effects: pain or hyperalgesia^{1,2,3}, diarrhoea^{4,5} and cough^{6,7}, that opiates are clinically used to lessen⁸. This antithesis extends to other effects of E prostaglandins and opiates⁹⁻¹³. Reports have also appeared of antagonism between morphine and E prostaglandins in some isolated intestinal preparations¹⁴⁻¹⁷ and in the release of adrenocorticotrophic hormone^{18,19}. Such considerations led us to examine whether morphine might inhibit some biochemical process of E prostaglandin (PGE) production or action at appropriate concentrations *in vitro*. The finding of Vane²⁰ that morphine does not inhibit biosynthesis of PGE₂ was confirmed, using rat brain homogenate with glutathione and hydroquinone as cofactors (S. A. Saeed, P. J. Gardiner and H. O. J. C., unpublished). Rat brain homogenate, however, converts adenosine triphosphate (ATP) to cyclic 3',5'-adenosine monophosphate (cyclic AMP) through the action

of adenylyl cyclase. We report that PGE₁ or PGE₂, but not PGE_{2a}, stimulated the formation of cyclic AMP from ATP in rat brain homogenate and that morphine-like drugs potently inhibited this stimulation without inhibiting the basal production of cyclic AMP in the absence of E prostaglandin.

Measurement of cyclic AMP formation

In preparing brain homogenate, Tris-HCl buffer (50 mM, pH 7.4), containing 250 mM sucrose, 1 mM MgCl₂, 25 mM KCl, 25 mM NaCl, 0.1 mM EDTA and 1.3 mM 2-mercaptoethanol, was used. The buffer was chilled to 0-2° C and operations were done whenever possible at this temperature. Rats (90-120 g) were decapitated, the whole brain was quickly removed, washed in buffer and transferred to fresh buffer. The brain was chopped and then gently homogenised in ten volumes of buffer in a Jencon glass homogeniser.

The formation of radioactive cyclic AMP from its labelled precursor, ³H-ATP, was measured at a total concentration of ATP in the reaction mixture of 1.16 μM. An excess of non-radioactive cyclic AMP was included in the reaction mixture to protect the newly formed radioactive cyclic AMP from degradation by phosphodiesterase²¹. Brain homogenate (0.05 ml) was incubated with 0.75 ml of a mixture containing Tris-HCl (pH 7.4, 50 mM); MgCl₂, 2 mM; KCl, 10 mM; NaCl, 10 mM; bovine serum albumin, 0.015%; cyclic AMP, 3 mM; ³H-ATP (21 Ci mmol⁻¹), 2.5 μCi;

unlabelled ATP, 1 μ M; with or without E prostaglandin (0.25–50 μ g per tube) and drug at concentrations stated in the text. The drugs used were (–)morphine sulphate, (–)heroin hydrochloride, ($\pm$)methadone hydrochloride, dextrorphan tartrate and (–)naloxone hydrochloride, the concentrations of drug being expressed as active base. Incubations were carried out in duplicate at 30° C for 20 min in a Gallenkamp shaking incubator and were terminated by immersion in boiling water for 3 min after the addition of 2 mM cyclic AMP and 2 mM ATP. After freezing and thawing, the tubes were centrifuged for 15 min at 2,500g. Reaction blanks consisted of incubation tubes containing previously boiled supernatant fractions.

The labelled cyclic AMP formed was isolated by chromatography on Dowex 50W-X4 columns followed by a double $\text{Ba}(\text{OH})_2\text{-ZnSO}_4$ precipitation²², care being taken that the pH of the final supernatants did not exceed 8 (ref. 23). The radioactivity contained in 1 ml aliquot of the final supernatants was measured in a Beckman LS-150 liquid scintillation spectrometer.

Blockade of PGE stimulation

In five experiments, addition of PGE_1 (50 or 25 μ g per 0.8 ml tube of incubate) increased cyclic AMP formation by 1.4–2.7 times the basal production in the control tubes (without added prostaglandin). In one experiment, PGE_1 was added to incubation tubes in amounts that were reduced tenfold. The stimulation by PGE_1 of cyclic AMP formation over that in control tubes was: 25 μ g PGE_1 , 2.7 $\times$; 2.5 μ g PGE_1 , 1.2 $\times$; 0.25 μ g PGE_1 , 0.92 $\times$. In both of two experiments, PGE_2 was about equal to PGE_1 in stimulant activity. In one experiment in which $\text{PGF}_{2\alpha}$ was tested at 25 μ g per tube, this prostaglandin was inactive.

In five of five experiments, morphine inhibited the stimulation by PGE_1 of cyclic AMP formation by rat brain homogenate, without inhibiting the basal production of cyclic AMP (Fig. 1). The potency of morphine was high and dose-related. For example, in the experiment of Fig. 1, the 50% inhibitory concentration (IC_{50}) was 1.46 $\mu\text{g ml}^{-1}$ (95% fiducial limits, 1.20–1.82) and the slope of the dose/response line based on percentage reduction of responses to PGE_1 per $\log_{10}$ concentration of morphine was 61.90 with s. e. ± 2.8 . This slope was highly significant ($P < 0.001$). In another experiment, in which PGE_2 was used instead of PGE_1 , morphine showed activity comparable with that against PGE_1 (IC_{50} , 2.03 $\mu\text{g ml}^{-1}$).

When the amount of PGE_1 used to stimulate cyclic AMP formation was reduced ten-fold, from 25 or 50 to 2.5 or 5 μ g per tube, the effectiveness of morphine was raised (Fig. 2). In this figure, based on two experiments, the mean slope of the line relating the inhibitory effect of a given concentration of morphine (0.57 $\mu\text{g ml}^{-1}$) to the $\log_{10}$ concentration of PGE_1 was -16.26 ± 3.71 , which was highly significant ($P < 0.005$).

In one experiment, 8 mM theophylline was added to the incubation mixture. In this experiment, stimulation by PGE_1 of cyclic AMP formation, which was 2.6 times that in the control, was within the usual range and morphine was effective in a dose-related way at the usual concentrations (0.57 and 5.7 $\mu\text{g ml}^{-1}$).

Heroin and methadone likewise inhibited the stimulation by PGE_1 of cyclic AMP formation by rat brain homogenate. In the experiment of Fig. 3, the IC_{50} values in $\mu\text{g ml}^{-1}$ were: heroin, 0.159 (limits 0.037–0.314); methadone, 10.6 (limits 6.53–21.8). The slopes of the dose/response lines, derived as for morphine, with standard errors, were: heroin, 49.57 ± 9.957 ($P < 0.02$); methadone, 69.62 ± 7.38 ($P < 0.005$). Neither slope differed significantly from that of morphine in Fig. 1. In two experiments, dextrorphan, at 50 or 600 $\mu\text{g ml}^{-1}$, was devoid of inhibitory activity.

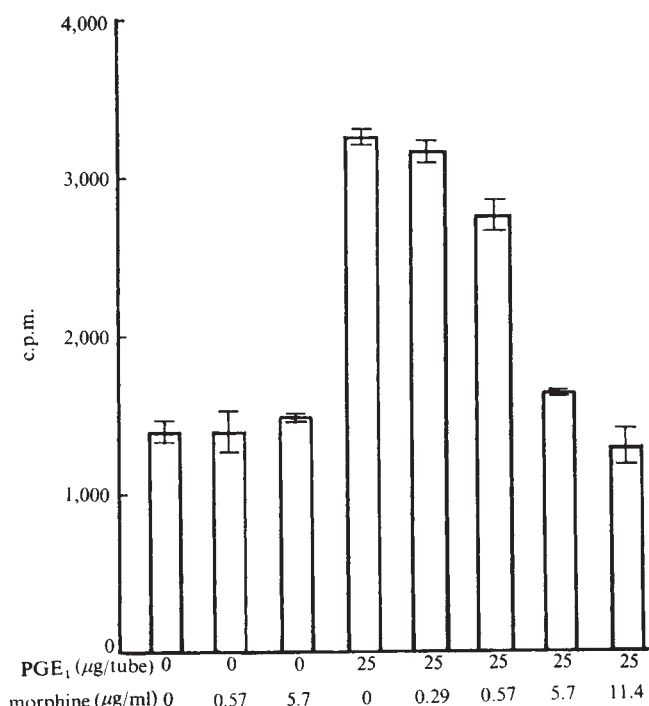


FIG. 1 Inhibition by morphine of the conversion of ^3H -ATP to ^3H -cyclic AMP stimulated by prostaglandin E_1 in rat brain homogenate. The amount of ^3H -cyclic AMP formed after incubation for 20 min, in the presence or absence of 25 μ g per tube of PGE_1 and various concentrations of morphine, is expressed in mean c.p.m. $\pm$ s.e.

We also tested whether naloxone antagonised the inhibitory effect of morphine on the stimulation by PGE_1 or PGE_2 of cyclic AMP formation. In both of two experiments, naloxone, at a molar ratio to morphine of 1:2, completely antagonised the effect of morphine. A feature of these experiments that will require further investigation was that naloxone alone also affected cyclic AMP formation.

In two preliminary experiments, using an analogous method, morphine also inhibited the stimulation by PGE_1 of cyclic AMP formation by homogenate of rat intestine. The IC_{50} was roughly 2 $\mu\text{g ml}^{-1}$.

Biochemical action and pharmacological effects

The stimulation by PGE_1 or PGE_2 of cyclic AMP formation by rat brain homogenate was probably due to the activation of an adenylyl cyclase; but, conceivably, it could have been due to inhibition of phosphodiesterase or of ATPase^{24,25}. That this effect of PGE_1 occurred in the presence of theophylline seems to rule out phosphodiesterase inhibition. Although the possibility that the effect was due to inhibition of ATPase seems slight and the activation of adenylyl cyclase by E prostaglandins is well documented, we prefer at this stage to speak of the stimulation of cyclic AMP formation rather than of adenylyl cyclase.

There seems little doubt that the interaction of E prostaglandins with the cyclic AMP system is a step in their mechanism of action in some cells. The above observations therefore raise the question: would inhibition of the stimulation by E prostaglandin of cyclic AMP formation in appropriate cells explain the mechanism of analgesic or other action of opiates? There are four lines of evidence bearing upon this question.

One line of evidence concerns the question of whether the occurrence and actions of E prostaglandins are such that their inhibition might explain some of the pharmacological effects of morphine. One such action of E prostaglandins is produc-

tion of pain or hyperalgesia; but this has so far been clearly demonstrated only in peripheral sites¹⁻³, whereas morphine is believed to act centrally²⁶⁻²⁸. There is some evidence, however, that in appropriate regions of the brain, E prostaglandins may also elicit pain or hyperalgesia. Thus, fever in which E prostaglandin is produced in the hypothalamus⁹, may be accompanied by severe headache or malaise. Again, in the rat, sodium salicylate, which inhibits E prostaglandin synthesis²⁰, lessens nociception generated by stimulation of the lateral hypothalamus with implanted electrodes²⁹. The mechanism whereby E prostaglandins might elicit or enhance pain centrally is uncertain; but they antagonise release or action of noradrenaline³⁰⁻³², which may exert an analgesic action³³⁻³⁵, and they may participate in acetylcholine release from nerve terminals¹⁷ and in conduction between neurones³⁶.

Because PGE₁ is thought to be "an ideal general mediator for raising body temperature"¹⁰, morphine might be expected to lower body temperature when this was being raised by production of E prostaglandins in the thermo-regulatory centres of the anterior hypothalamus. Morphine seems seldom to have been critically tested as an antipyretic while this mechanism was active; but we have traced one report in which this may have been done. In rats kept in a cold environment (5° C), injection of morphine, either 50 µg into the anterior hypothalamus or 50 mg kg⁻¹ intraperitoneally, did indeed lower body temperature, by a mean of 5-6° C (ref. 37). Whereas, when rats were maintained at 32° C, and PGE production in the thermoregulatory centres was presumably in abeyance, morphine by either route raised mean body temperature, by 1-2° C. In a thermoneutral environment (24° C) morphine had little effect in these experiments; but other investigators have found it to be mainly hypothermic when injected intracerebrally or systemically in the rat¹³. Tolerance can develop quickly to this hypothermic effect of morphine, revealing a hyperthermic component.

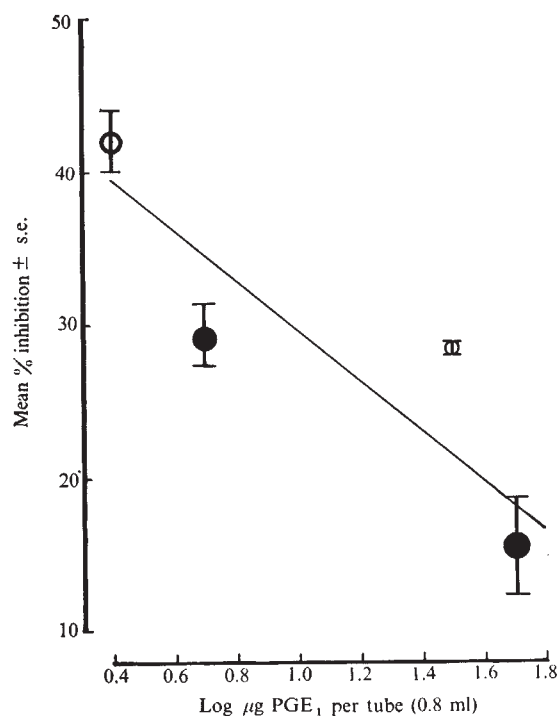


FIG. 2 Increased inhibition by morphine of the stimulation of cyclic AMP formation when the concentration of PGE₁ was reduced tenfold. The inhibitory effects in two experiments of one concentration of morphine (0.57 µg ml⁻¹) on the response to PGE₁ of rat brain homogenate are plotted against log concentration of prostaglandin.

Blockade of E prostaglandin in intestinal tissue might suppress diarrhoea, because E prostaglandin, which seems, with cyclic AMP to be involved in diarrhoea induced by ionising radiation³⁸ or toxins^{39,40}, increased fluid accumulation in the intestinal lumen^{4,5}.

A second line of evidence concerns antagonism between morphine, on the one hand, and cyclic AMP or PGE, on the other. If morphine acted as an analgesic by inhibiting PGE-activated cyclic AMP formation, we might expect that its potency would be reduced by administration of cyclic AMP. That cyclic AMP does indeed lessen the antinociceptive effect of morphine in the mouse and rat has been demonstrated⁴¹. There is evidence also that morphine can antagonise some effects of E prostaglandins on intestinal muscle¹⁵⁻¹⁷ and on the hypothalamus^{18,19}; and it has recently been proposed that morphine antagonises the mediation by E prostaglandins of acetylcholine release from guinea pig ileum¹⁷.

A third line of evidence is provided by our finding that the effectiveness of heroin, morphine and methadone, the ineffectiveness of the inactive stereoisomer, dextrorphan, and the antagonism of morphine by naloxone form a pattern consistent with the known pharmacological relationships of these drugs. Why the relative potencies of heroin, morphine and methadone against stimulation by PGE₁ of cyclic AMP formation slightly differ from their relative antinociceptive potencies *in vivo* could be explained in terms of the absorption and metabolism of these drugs, as discussed below.

Fourth, there is the question of how far the concentrations of opiates that inhibit the PGE stimulation of cyclic AMP formation *in vitro* correspond with those needed in tissues for antinociceptive effects *in vivo*. The antinociceptive ED₅₀ values of morphine obtained in the rat were 0.87 mg kg⁻¹ subcutaneously in the abdominal constriction test and 6.08 mg kg⁻¹ subcutaneously in the tail-pressure test⁴². After 5 mg kg⁻¹ morphine subcutaneously, values of 0.25 µg g⁻¹ and of 0.179 µg g⁻¹ were obtained for whole brain and 0.58 µg g⁻¹ for the hypothalamic area^{43,44}. In the experiment in Fig. 1, the IC₅₀ value of morphine for inhibiting the stimulation by PGE₁ of cyclic AMP formation in brain homogenate was 1.46 µg ml⁻¹. This, therefore, does not much exceed the value obtained for the hypothalamic concentration *in vivo* after a dose of the approximate ED₅₀ of morphine in the tail-pressure test. That morphine was more effective *in vitro* when less PGE₁ was used to stimulate cyclic AMP formation allows flexibility in matching *in vitro* and *in vivo* concentrations. Further flexibility could arise from the possibility that the drugs used might undergo some metabolism by rat brain homogenate *in vitro*.

Heroin is about three times as potent as morphine in the abdominal constriction test in the mouse⁴⁵, but its true analgesic potency is masked by its rapid conversion in the body to morphine and 6-monoacetylmorphine⁴⁶. In the guinea pig isolated ileum, heroin was about twice as active as morphine in a test that correlates well with analgesic activity⁴⁷. In antinociceptive tests in mouse, rat and rabbit, methadone is slightly more potent than morphine, but methadone is much better absorbed into the brain⁴⁸. In the rabbit, for example, to provide an equal antinociceptive effect, a concentration of methadone approximately thirty times higher than that of morphine is needed in the brain²⁸. The concentrations of heroin, morphine and methadone that inhibit the stimulation by PGE₁ of cyclic AMP formation *in vitro* thus probably correspond with the concentrations of drug or active derivative that are effective *in vivo*. Moreover, the relative potencies of (–) morphine and (±) methadone and the ineffectiveness of dextrorphan are consistent with their affinities for the opiate receptor of rat brain homogenate⁴⁸.

Thus, four lines of evidence support this analysis of opiate action. First, some actions of E prostaglandins are such that their antagonism would explain some of the main

pharmacological effects of opiates. Second, cyclic AMP antagonises the antinociceptive effect of morphine and morphine antagonises some effects of E prostaglandins. Third, the relationship between heroin, morphine, methadone, dextrorphan and naloxone in our experiments *in vitro* largely corresponds with their pharmacological relationship

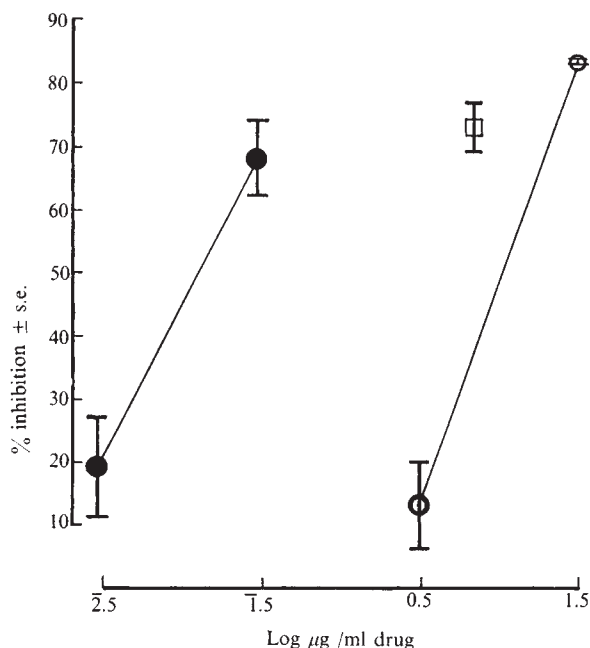


FIG. 3 Inhibition by heroin (●), morphine (□) and methadone (○) of the stimulation by PGE_1 of cyclic AMP formation in rat brain homogenate.

in vivo. Fourth, the concentrations at which heroin, morphine and methadone inhibit PGE_1 *in vitro* roughly correspond with effective concentrations in brain *in vivo*. We therefore propose that the ability of opiates to inhibit the stimulation by E prostaglandins of cyclic AMP formation in rat brain homogenate, presumably by inhibiting stimulation of a neuronal adenylyl cyclase, represents a biochemical mechanism that could account for the analgesic and allied effects of these drugs. Such a mechanism could accommodate interactions of opiates with other humoral messengers. If this is how opiates act, then tolerance and dependence, which arise from the agonist action of opiates⁴⁹, are enhanced by cyclic AMP⁵⁰ and are partly blocked by indomethacin⁵¹, may represent a compensating hypertrophy of a part of the inhibited PGE -cyclic AMP mechanism.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Possible observation of tachyons associated with extensive air showers

SEVERAL searches¹⁻⁴ have been made for tachyons using either laboratory particle sources or high energy cosmic rays. Effects associated with their supposed characteristic mass and velocity have been searched for but so far no positive evidence has been reported. As has been repeatedly pointed out, however, the goal of these searches is so important that all possible avenues should be fully investigated. We report here apparently positive results from a pilot search for tachyons associated with cosmic ray showers of energy about 2×10^{15} eV.

The first interaction of a primary cosmic-ray nucleon occurs at a typical altitude of 20 km (ref. 5). Further interactions result in a cascade of relativistic particles travelling with speeds close to that of light (c). Thus, most of the particles in this extensive air shower (EAS) arrive at sea level with a time spread of only a few nanoseconds⁶. If any shower particles are produced with velocities greater than c , they should be observable in the time interval up to 20 km/ c (60 μ s) before the arrival of the shower front. The precise time depends on their velocity and production altitude. A pilot experiment has been conducted to search for particles arriving within this time period. In this experiment, the EAS which were studied had energies some two orders of magnitude higher than in previously reported work⁴.

A plastic scintillator was used to detect the particles. It is not clear what interactions a tachyon might have with the atmosphere or the scintillator. The present search was made assuming that tachyons are produced in EAS interactions at heights between 20 km and 400 m (800 kg m⁻² and 10,000 kg m⁻²) and have a sufficiently long absorption length for some to reach the detector. Detection might be accomplished by direct interaction of the tachyons with the scintillator or through the production of secondary particles which interact with the detector. It is not necessary that a single tachyon should produce a large response in the scintillator (as, for instance, a charged relativistic particle would). In principle, provided that observations are initiated by the detection of EAS, it is possible to sum results from many observations so that small non-random effects can be observed.

The chief experimental difficulty with this procedure is that if a recording device is triggered by the arrival of an EAS, it will be too late to observe the tachyon unless substantial signal delays are inserted. This was overcome in the present case by the use of a digital transient recorder (Biomation International, Palo Alto, California; model 610B) which enabled us to trigger our recording system from an EAS and then examine the signal from the particle detector recorded prior to the arrival of the trigger. The device continually samples and digitises (to six bit accuracy) the output of the particle detector. Two hundred and fifty-six words of this digitised data are stored in a shift register which is continually updated. Thus, when a trigger is received, the previous 256 words of data are in store (in our case representing 128 μ s) and can be output at leisure. Output was to a chart recorder after digital to analogue conversion. In this mode, the recorder only outputs 228 words (114 μ s).

The EAS trigger was obtained from the fast timing part of the air shower array at the Buckland Park field station of the University of Adelaide. Five 1 m square plastic scintillators, 50 mm thick, were used, in a square array of side 30 m, one scintillator being at each corner with one at the centre. Each scintillator was viewed with a Philips XP1040 photomultiplier and the arrival of an EAS was detected by a coincidence between the centre detector and any three of the outer detectors with a resolving time of 150 ns. Thus, air showers were detected from a cone about the zenith, with half angle of about 35°. The mean rate of showers was one per 500 s, corresponding to a minimum shower energy of about 2×10^{15} eV. In addition, one of the corner scintillators was also viewed by an RCA 8055 photomultiplier connected to a charge sensitive preamplifier, the output of which was the signal recorded by the transient recorder. The impulse response of the system had a width of 1.7 μ s at half maximum ensuring that a sampling interval of 0.5 μ s gave an acceptable reconstruction of the waveform on digital to analogue conversion.

Data from a total of 1,307 air showers, detected between February and August, 1973, have been analysed. The aim of the analysis was to demonstrate whether or not non-random effects were observable immediately preceding an EAS and for this reason a simple analysis procedure was employed. The time (with respect to the arrival of each EAS) of the largest excursion of the amplified photomultiplier output was noted. If there was doubt as to which of a number of pulses was the largest, all the apparently equal pulses were included. In practice, approximately 4% of the events had their two largest pulses sufficiently close in amplitude to cause ambiguity. In order to check on observer bias in assigning relative pulse heights, approximately 600 events were re-read by an independent chart reader. Some 3% of the events had the assignment of the largest pulse changed but no systematic bias was found.

The position of the shower front on the output trace can be adjusted; thus the time interval available for analysis is dependent on the exact setting of the transient recorder. For 1,176 of the events the record extended beyond 105 μ s before the air shower arrival. Figure 1a shows a histogram of the positions of the largest pulses for these events. The figure suggests that the distribution of the largest pulses is not uniform and a χ^2 test on the data indicates that there is only 1% chance that the data is from a uniform distribution.

In order to include data from all 1,307 recorded events it is necessary to limit the period of analysis to 97.5 μ s before the shower arrival. The resulting histogram is shown in Fig. 1b. A χ^2 test on this data shows less than 0.1% probability that the data is from a uniform distribution. In addition, since if tachyons are observed one might expect their arrival times to be spread over more than one 7.5 μ s bin, one can also test to see whether there are specific time regions contributing excessively to the non uniformity or whether the large χ^2 is due to a more or less random time distribution of excesses. This problem has been discussed by David⁷ who noted that a calculation of χ^2 involved the squaring of deviations from a hypothetical set and that independent information on the sign of the deviations was ignored. An examination of the distribution of bins above and below the mean shows that the probability that our data are selected from a uniform distribution is less than 1%, on the basis of a non-parametric run test.

The data were obtained in 12 runs containing between 70 and 180 events each and the data were tested for the possibility of non-random variations between runs. This was done

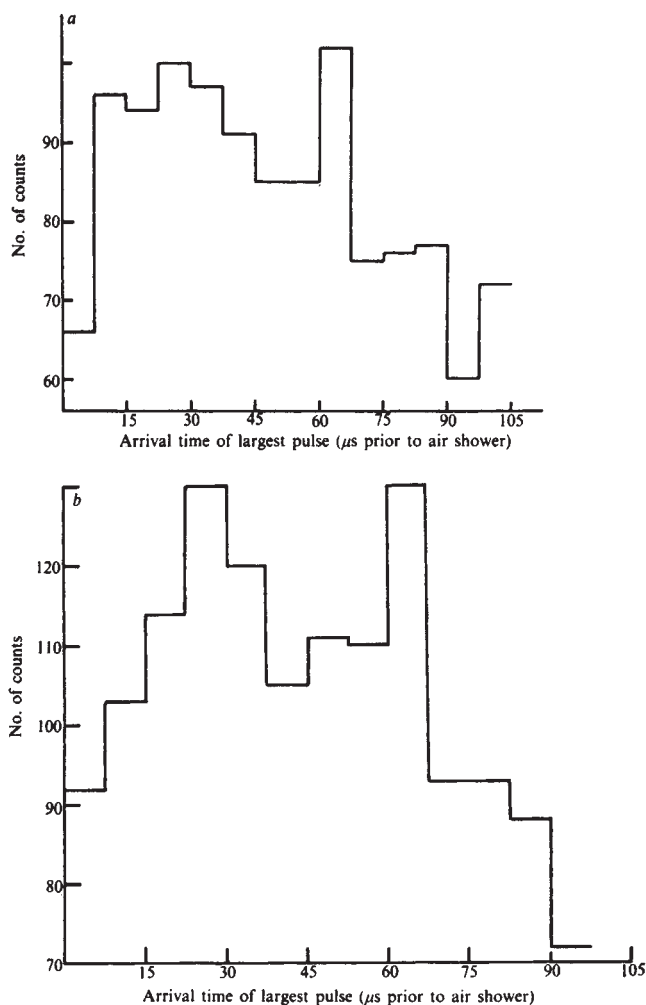


FIG. 1 The time distribution of largest photomultiplier pulse in the period prior to the arrival of an extensive air shower; *a*, data from 1,176 air showers in 14 bins of width 7.5 μ s; *b*, data from all 1,307 showers observed in 13 bins of width 7.5 μ s.

by comparing data obtained from individual runs with the final distribution. Taking deviations of each bin of each run from the normalised final distribution gave a χ^2 indicating less than a 10% probability that the individual runs were not

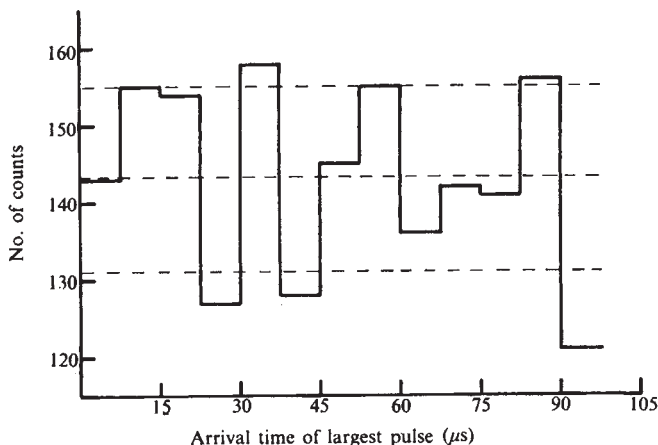


FIG. 2 The time distribution of largest photomultiplier pulses in 1,839 random samples of output in 13 bins of width 7.5 μ s.

randomly selected subsets of the total data.

The experiment has the unique quality that spurious pickup in the electronics is most unlikely to affect the result since pickup must occur at or after the arrival of the shower front, thus arriving after recording and storage of the data. It is arguable that the arrival of an air shower could generate interference in the recorder memory. If this were so, unless

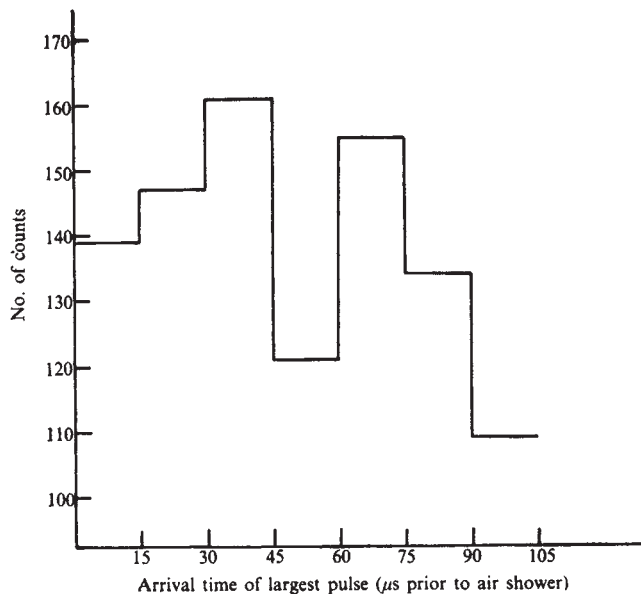


FIG. 3 The time distribution of largest photomultiplier pulses in the 105 μ s period prior to 978 extensive air showers. The data was recorded at half the sampling rate of that in Figs 1 and 2 and analysed into 7 bins of width 15 μ s.

only the least significant bit were affected, a discontinuity in the trace would be expected after digital to analogue conversion. In more than 3,000 events no such effect was observed. In addition, grossly overloading the input amplifier with a pulse was found to have no effect on stored data.

In case there was some other form of pickup or of observer bias, we have triggered our coincidence system with artificial pulses and repeated the analysis on an apparently random sample of photomultiplier output; 1839 events were analysed in the same way as the air shower triggered data indicating on the basis of a χ^2 test a probability of about 40% that these test results were from a uniform distribution. The data are presented in Fig. 2. The probability that this test data and the EAS data are from the same distribution is less than one in 10^4 on the basis of a χ^2 test.

We have also operated our transient recorder at one half the previous sampling rate (with a lower bandwidth) and taken results over a similar range to that described above. These data from a further 972 showers were analysed into seven coarser bins (15 μ s wide) but otherwise a similar analysis technique was employed. The resulting distribution shown in Fig. 3 seems to exhibit the same broad features as Fig. 1. We use this as further evidence that the equipment and analysis technique are largely free from bias since the equipment was now operated in a rather different manner.

Ramana Murthy⁴ has reported an unsuccessful search for tachyons in EAS. Two detection techniques were employed, in one of which a liquid scintillator was used and a search made for particles immediately prior to EAS. This experiment is rather similar to the one described above but differs in a number of important respects. In order to avoid the use of long analogue delays, his measurements were initiated by the detection of single charged particles and a search was made for EAS following within 19.2 μ s. This technique is much less efficient than ours since only 1 in 250 potential tachyons was followed by an EAS within the time of interest. Also, potential tachyons have the additional constraint that

they must produce a response in the scintillator comparable with that produced by a conventional charged particle. As mentioned earlier, the EAS studied by Ramana Murthy were almost two orders of magnitude less energetic than those used by us and also a much smaller time range was examined. Since the peak in our distribution occurs after the 18 μ s time interval investigated by Ramana Murthy, the results cannot be directly compared. But a statistical examination of his published data using David's technique indicates that there is less than 5% probability that the data is from a uniform distribution. We note that, subjectively, the observed distribution seems to rise in the period prior to 13 μ s before the shower arrival. This is not inconsistent with our observations.

It is possible than an explanation may be found for these results without invoking the existence of tachyons. A. G. Gregory has pointed out to us that fission or spallation in the interstellar medium or production of associated particles at the source might account for the correlated arrival of cosmic rays. We note, however, that unless particles are produced with closely similar rigidity and velocity vectors they are unlikely to remain associated for long in the interstellar or source magnetic fields. A closely related problem has been discussed by Weekes⁸ in connection with pulsar periodicities in cosmic-ray arrival times.

We conclude that we have observed non-random events preceding the arrival of an extensive air shower. Being unable to explain this result in a more conventional manner, we suggest that this is the result of a particle travelling with an apparent velocity greater than that of light.

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Black hole explosions?

QUANTUM gravitational effects are usually ignored in calculations of the formation and evolution of black holes. The justification for this is that the radius of curvature of space-time outside the event horizon is very large compared to the Planck length $(G\hbar/c^3)^{1/2} \approx 10^{-33}$ cm, the length scale on which quantum fluctuations of the metric are expected to be of order unity. This means that the energy density of particles created by the gravitational field is small compared to the space-time curvature. Even though quantum effects may be small locally, they may still, however, add up to produce a significant effect over the lifetime of the Universe $\approx 10^{17}$ s which is very long compared to the Planck time $\approx 10^{-43}$ s.

The purpose of this letter is to show that this indeed may be the case: it seems that any black hole will create and emit particles such as neutrinos or photons at just the rate that one would expect if the black hole was a body with a temperature of $(\kappa/2\pi)(\hbar/2k) \approx 10^{-6} (M_\odot/M)K$ where κ is the surface gravity of the black hole¹. As a black hole emits this thermal radiation one would expect it to lose mass. This in turn would increase the surface gravity and so increase the rate of emission. The black hole would therefore have a finite life of the order of $10^{71} (M_\odot/M)^{-3}$ s. For a black hole of solar mass this is much longer than the age of the Universe. There might, however, be much smaller black holes which were formed by fluctuations in the early Universe². Any such black hole of mass less than 10^{15} g would have evaporated by now. Near the end of its life the rate of emission would be very high and about 10^{30} erg would be released in the last 0.1 s. This is a fairly small explosion by astronomical standards but it is equivalent to about 1 million 1 Mton hydrogen bombs.

To see how this thermal emission arises, consider (for simplicity) a massless Hermitean scalar field ϕ which obeys the covariant wave equation $\phi;_{ab}g^{ab} = 0$ in an asymptotically flat space time containing a star which collapses to produce a black hole. The Heisenberg operator ϕ can be expressed as

$$\phi = \sum_i \{f_i a_i + \bar{f}_i a_i^+\}$$

where the f_i are a complete orthonormal family of complex valued solutions of the wave equation $f_{i,ab}g^{ab} = 0$ which are asymptotically ingoing and positive frequency—they contain only positive frequencies on past null infinity I^- ^{3,4,5}. The position-independent operators a_i and a_i^+ are interpreted as annihilation and creation operators respectively for incoming scalar particles. Thus the initial vacuum state, the state containing no incoming scalar particles, is defined by $a_i|0\rangle = 0$ for all i . The operator ϕ can also be expressed in terms of solutions which represent outgoing waves and waves crossing the event horizon:

$$\phi = \sum_i \{p_i b_i + \bar{p}_i b_i^+ + q_i c_i + \bar{q}_i c_i^+\}$$

where the p_i are solutions of the wave equation which are zero on the event horizon and are asymptotically outgoing, positive frequency waves (positive frequency on future null infinity I^+) and the q_i are solutions which contain no outgoing component (they are zero on I^+). For the present purposes it is not necessary that the q_i are positive frequency on the horizon even if that could be defined. Because fields of zero rest mass are completely determined by their values on I^- , the p_i and the q_i can be expressed as linear combinations of the f_i and the $\bar{f}_i$:

$$p_i = \sum_j \{\alpha_{ij} f_j + \beta_{ij} \bar{f}_j\} \quad \text{and so on}$$

The β_{ij} will not be zero because the time dependence of the metric during the collapse will cause a certain amount of mixing of positive and negative frequencies. Equating the two expressions for ϕ , one finds that the b_i , which are the annihilation operators for outgoing scalar particles, can be expressed as a linear combination of the ingoing annihilation and creation operators a_i and a_i^+

$$b_i = \sum_j \{\bar{\alpha}_{ij} a_j - \bar{\beta}_{ij} a_j^+\}$$

Thus when there are no incoming particles the expectation value of the number operator $b_i^* b_i$ of the i th outgoing state is

$$\langle 0_- | b_i^* b_i | 0_- \rangle = \sum_j |\beta_{ij}|^2$$

The number of particles created and emitted to infinity in a gravitational collapse can therefore be determined by calculating the coefficients β_{ij} . Consider a simple example in which

the collapse is spherically symmetric. The angular dependence of the solution of the wave equation can then be expressed in terms of the spherical harmonics Y_{lm} and the dependence on retarded or advanced time u, v can be taken to have the form $\omega^{-1/2} \exp(i\omega u)$ (here the continuum normalisation is used). Outgoing solutions $p_{lm\omega}$ will now be expressed as an integral over incoming fields with the same l and m :

$$p_\omega = \int \{\alpha_{\omega\omega'} f_{\omega'} + \beta_{\omega\omega'} \bar{f}_{\omega'}\} d\omega'$$

(The lm suffixes have been dropped.) To calculate $\alpha_{\omega\omega'}$ and $\beta_{\omega\omega'}$, consider a wave which has a positive frequency ω on I^+ propagating backwards through spacetime with nothing crossing the event horizon. Part of this wave will be scattered by the curvature of the static Schwarzschild solution outside the black hole and will end up on I^- with the same frequency ω . This will give a $\delta(\omega - \omega')$ behaviour in $\alpha_{\omega\omega'}$. Another part of the wave will propagate backwards into the star, through the origin and out again onto I^- . These waves will have a very large blue shift and will reach I^- with asymptotic form

$$C\omega^{-1/2} \exp\{-i\omega\kappa^{-1} \log(v_0 - v) + i\omega v\} \text{ for } v < v_0$$

and zero for $v \geq v_0$, where v_0 is the last advanced time at which a particle can leave I^- , pass through the origin and escape to I^+ . Taking Fourier transforms, one finds that for large ω' , $\alpha_{\omega\omega'}$ and $\beta_{\omega\omega'}$ have the form:

$$\begin{aligned} \alpha_{\omega\omega'} &\approx C \exp[i(\omega - \omega)v_0](\omega'/\omega)^{1/2} \\ &\quad \cdot \Gamma(1 - i\omega/\kappa)[-i(\omega - \omega')]^{-1+i\omega/\kappa} \\ \beta_{\omega\omega'} &\approx C \exp[i(\omega + \omega)v_0](\omega'/\omega)^{1/2} \\ &\quad \cdot \Gamma(1 - i\omega/\kappa)[-i(\omega + \omega')]^{-1+i\omega/\kappa}. \end{aligned}$$

The total number of outgoing particles created in the frequency range $\omega \rightarrow \omega + d\omega$ is $d\omega \int_{\omega'} |\beta_{\omega\omega'}|^2 d\omega'$. From the above expression it can be seen that this is infinite. By considering outgoing wave packets which are peaked at a frequency ω and at late retarded times one can see that this infinite number of particles corresponds to a steady rate of emission at late retarded times. One can estimate this rate in the following way. The part of the wave from I^+ which enters the star at late retarded times is almost the same as the part that would have crossed the past event horizon of the Schwarzschild solution had it existed. The probability flux in a wave packet peaked at ω is roughly proportional to $\int_{\omega'} \omega_2' \{|\alpha_{\omega\omega'}|^2 - |\beta_{\omega\omega'}|^2\} d\omega'$ where $\omega_2' \gg \omega_1' \gg 0$. In the expressions given above for $\alpha_{\omega\omega'}$ and $\beta_{\omega\omega'}$, there is a logarithmic singularity in the factors $[-i(\omega - \omega')]^{-1+i\omega/\kappa}$ and $[-i(\omega + \omega')]^{-1+i\omega/\kappa}$. Value of the expressions on different sheets differ by factors of $\exp(2\pi n\omega\kappa^{-1})$. To obtain the correct ratio of $\alpha_{\omega\omega'}$ to $\beta_{\omega\omega'}$ one has to continue $[-i(\omega + \omega')]^{-1+i\omega/\kappa}$ in the upper half ω' plane round the singularity and then replace ω' by $-\omega'$. This means that, for large ω' ,

$$|\alpha_{\omega\omega'}| = \exp(\pi\omega/\kappa) |\beta_{\omega\omega'}|$$

From this it follows that the number of particles emitted in this wave packet mode is $(\exp(2\pi\omega/\kappa) - 1)^{-1}$ times the number of particles that would have been absorbed from a similar wave packet incident on the black hole from I^- . But this is just the relation between absorption and emission cross sections that one would expect from a body with a temperature in geometric units of $\kappa/2\pi$. Similar results hold for massless fields of any integer spin. For half integer spin one again gets a similar result except that the emission cross section is $(\exp(2\pi\omega/\kappa) + 1)^{-1}$ times the absorption cross section as one would expect for thermal emission of fermions. These results do not seem to depend on the assumption of exact spherical symmetry which merely simplifies the calculation.

Beckenstein⁶ suggested on thermodynamic grounds that some multiple of κ should be regarded as the temperature of a black hole. He did not, however, suggest that a black hole could emit particles as well as absorb them. For this reason Bardeen, Carter and I considered that the thermodynamical similarity between κ and temperature was only an analogy. The present result seems to indicate, however, that there may be more to it than this. Of course this calculation ignores the back reaction of the particles on the metric, and quantum fluctuations on the metric. These might alter the picture.

Further details of this work will be published elsewhere. The author is very grateful to G. W. Gibbons for discussions and help.

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Absorption and emission by interstellar CH at 9 cm

RYDBECK, Ellder and Irvine¹ have recently detected the 9-cm lines of the $^2\Pi_{1/2}$, $J = 1/2$ doublet of interstellar CH. The $F = 1 \rightarrow 1$ transition at 3,335.475 MHz was observed in emission in a wide range of galactic sources ranging from dark clouds to the spiral arms in front of Cassiopea A. The two satellite transitions $F = 0 \rightarrow 1$ (at 3,263.788 MHz) and $F = 1 \rightarrow 0$ (at 3,349.185 MHz) were also observed in emission in several sources.

We have observed the 3,335.475 MHz transition of CH in several southern galactic sources. In RCW38 this line is seen in absorption, while the two satellite lines are seen in emission. In several sources the distribution of CH is found to be extended.

The observations were made on December 10 and 11, 1973, with the Parkes 64-m telescope equipped with a 9-cm parametric amplifier having a noise temperature of 150 K. The telescope beam at 9 cm is 6 arc min. The receiver output was analysed by a 512-channel digital correlator producing a spectral resolution of 19.5 kHz.

The Onsala observations¹ of CH emission at 3,335.475 MHz from Cloud 2 and W12 were confirmed. In Cloud 2 we measured an antenna temperature similar to that found with the Onsala 25-m telescope. For W12 it was 0.23 K, about 50% greater than the Onsala value of 0.15 K; however, at a position 5 arc min south (where the continuum intensity had fallen to one seventh of its peak value) the line signal had decreased by only 30%. A similar situation occurred in RCW36. Thus the CH distribution is considerably more extended than the continuum for these HII regions; in Cloud 2 it must be comparable with the 16 arc min beam of the Onsala 25-m telescope.

In RCW38 the 3,335.475 MHz CH line was observed in absorption (Fig. 1); this is the first case found of absorption by CH. But the two satellite lines at 3,263.788 and 3,349.185 MHz appear in emission, with an antenna temperature of 1.3 K for the former. The pattern of main line absorption and satellite line emission indicates substantial departures from thermodynamic equilibrium in the population distribution.

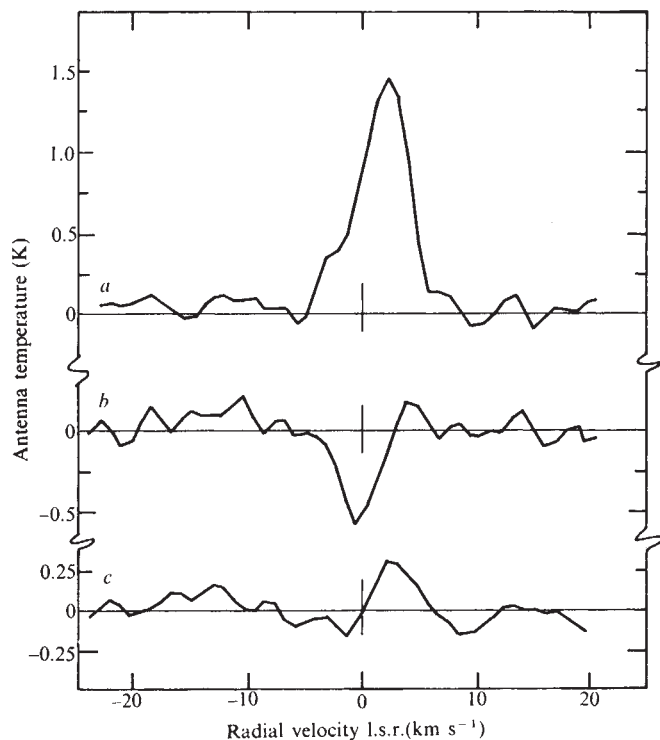


FIG. 1 9-cm lines of the $^2\Pi_{1/2}$, $J = 1/2$, CH Λ doublet observed in RCW38. Channel spacing is 10 kHz $\equiv$ 2.1 km s $^{-1}$. The local standard of rest radial velocities are based on the rest frequencies determined by Rydbeck *et al.*¹, a, $F = 0 \rightarrow 1$ at $3,263.788 \pm 0.010$ MHz; b, $F = 1 \rightarrow 1$ at $3,335.475 \pm 0.010$ MHz; c, $F = 1 \rightarrow 0$ at $3,349.185 \pm 0.010$ MHz.

A comparison of the CH profiles for RCW38 in Fig. 1 with the 18-cm OH profiles in Fig. 2 (from ref. 2) shows that the CH and OH profiles must contain at least two velocity components. The higher velocity component appears in absorption in OH at 1,665, 1,667 and 1,720 MHz, and in emission in OH at 1,612 MHz, and in CH at 3,264 and 3,349 MHz (and perhaps at 3,335 MHz). The lower velocity component appears in absorption on all four OH lines and in CH at 3,335 MHz, while it is in emission in CH at 3,264 MHz. Detailed comparison of the CH and OH profiles is restricted by the present limited accuracy of the rest frequencies deduced by Rydbeck *et al.*¹ (± 10 kHz, equivalent to ± 1 km s $^{-1}$ in radial velocity).

The CH population distribution in RCW38 required to explain the substantial departures from LTE could be similar to that of the $^2\Pi_{1/2}$, $J = 1/2$ levels of OH in Sagittarius B2 advanced by Gardner and Ribes.³ With their model, maser amplification of the $F = 0 \rightarrow 1$ transition occurs; as it is the region in front of the continuum source which is sampled we would expect the CH emission profile to resemble that for the OH absorption, as it does; we also find that the $F = 0 \rightarrow 1$ line intensity decreases in proportion to the continuum when the beam is pointed 6 arc min north of the source maximum. The model indicates that there is no inversion of the $F = 1 \rightarrow 1$ transition; since we observe an absorption

line the excitation temperature for this transition must be less than the peak brightness temperature in RCW38, which is about 1,500 K (from the emission measure of ref. 4). For the $F = 1 \rightarrow 0$ transition there might not be inversion,

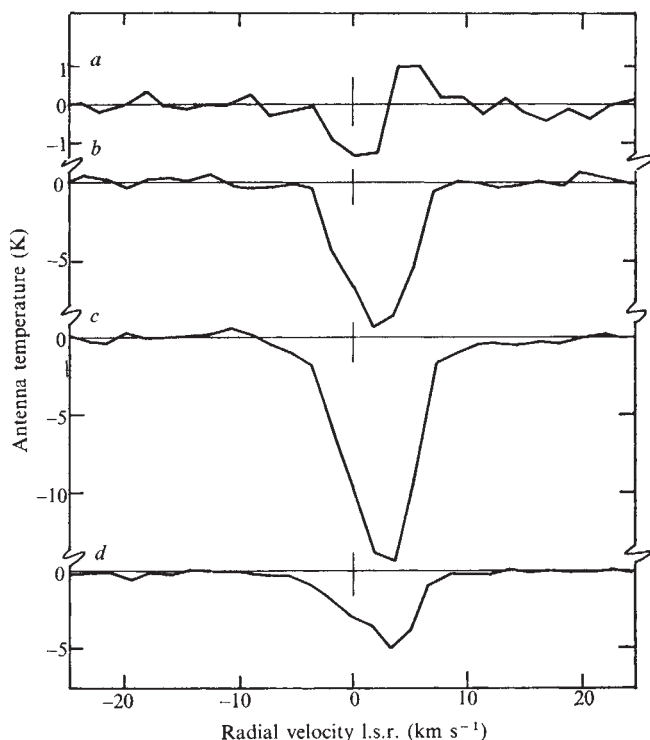


FIG. 2 18-cm lines of the $^2\Pi_{3/2}$, $J = 3/2$ OH Λ doublet observed in RCW38 (from Manchester *et al.*²). Filter bandwidth is 10 kHz $\equiv$ 1.8 km s $^{-1}$. a, 1,612 MHz; b, 1,665 MHz; c, 1,667 MHz; d, 1,720 MHz.

although the excitation temperature is probably higher than for $F = 1 \rightarrow 1$ since the line is observed in emission. Such a population distribution could result from a process of formation or excitation which preferentially filled the upper $F = 0$ level.

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Observations of Sgr A at 160 MHz with 1.9 arc min resolution

THE radio source Sgr A, which is located at or near the galactic centre, has now been observed with high resolution (<1 arc min) at several frequencies between 327 and 15 GHz¹⁻⁴. Downes and Martin³ have suggested that two main components are present between 1,400 and 5,000 MHz: Sgr A-West, identified with the galactic nucleus, coincides with the position of the $2.2\ \mu\text{m}$ source of Becklin and Neugebauer⁵. Its size is about 45 arc s and it may contain smaller scale structure^{3,4}. Sgr A-East, about 1.5 arc min to the east, is larger (~ 2.5 arc min) and has a steeper spectrum³ so that it dominates at low frequencies.

Here we extend the high resolution observations of Sgr A down to 160 MHz. This is about the lowest frequency at which observations are possible because the exponential fall-off in flux makes the source undetectable below ~ 120 MHz.

The 160 MHz observations were made with the radio-heliograph at Culgoora, NSW^{6,7}. This instrument has a beam diameter at the zenith of 1.9 arc min (Sgr A passes within 1 arc deg of the zenith). Each observation consisted of a series of about 10 drift scans made with 16 heliograph beams which were arranged to straddle the declination of the source. The 10 drift scans were averaged and used to produce a map of the brightness distribution over an area of 30×17 arc min. Ten such maps, obtained on different occasions and at a variety of hour angles, were superimposed to produce the final map. This procedure minimises the effects due to noise and the presence of other sources in the grating lobes of the radioheliograph. The beam-forming procedure in the heliograph is a switched system in which the area of the sky within about 4 arc deg of the beam is used as a reference. Thus the heliograph does not respond to large scale galactic structure and the temperature of the base level underlying a discrete source is unknown.

Figure 1 shows the brightness distribution of Sgr A obtained by this procedure. There seems to be a small compact source, possibly unresolved, which is apparently superimposed on an extended component about 8 arc min in diameter. The position of the peak, RA 17 h 42 min 33.6 ± 1.0 s, dec. $-28^\circ 58' 40'' \pm 20''$ (1950), is almost exactly the same as that in Little's⁸ map at 408 MHz. This compact source probably corresponds to Sgr A-East.

Krishna *et al.*² have observed Sgr A at 327 MHz using lunar occultations which gave an effective resolution of $\lesssim 1$

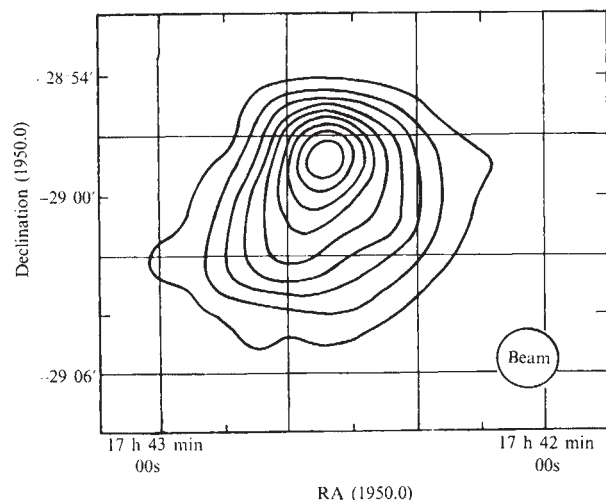


FIG. 1 Contour diagram of Sgr A at 160 MHz. The peak main beam brightness temperature, T_p , is 6.3×10^4 K. Contour levels are 10%, 20%, . . . 90% of T_p .

arc min. They claim to have detected Sgr A-West (they refer to it as component "A" but give no flux density) and have suggested that Sgr A-East contains a compact component ("S") which is slightly weaker than Sgr A-West and separated from it by about 1.5 arc min. We have examined each of our individual maps at 160 MHz (as well as the combined map of Fig. 1) and Little's map at 408 MHz (where the resolution is 2.9 arc min) and can find no indication of Sgr A-West or of any splitting into two subsources. If Sgr A-West is no stronger at 160 and 408 MHz than reported by Downes and Martin³ at 1,400, 2,700 and 5,000 MHz (30 f.u.), then we would not expect to see it. We conclude that the presence of Sgr A-West at frequencies lower than 1,400 MHz is not confirmed.

The extended component seen on Fig. 1 probably corresponds to the extended component (designated "E") of Krishna *et al.*², but its shape is considerably different. This component is also prominent on the 408 MHz map of Little⁸, where its shape is generally similar to that on Fig. 1, but the brightness falls off more slowly to the north and the ridge line to the south-east is less prominent than at 160 MHz, probably because of the somewhat lower resolution.

The measured flux density of Sgr A at 160 MHz is 114 ± 16 f.u. (16 f.u. is twice the expected error derived from 10 independent measurements.) This value includes the contributions of both the extended and compact components. On several occasions we have repeated Dulk's⁹ 80 MHz observations of Sgr A. We have made no consistent detections of the source and the upper limit of 5 f.u. remains unchanged.

Figure 2 shows most of the published measurements of the flux density of Sgr A. The measurements of the total flux density between about 400 MHz and 20 GHz can be fitted by a power-law spectrum with spectral index ~ -0.25 . As seen on Fig. 2, however, a more consistent interpretation is obtained if we assume that Sgr A-West has a nearly flat spectrum and the remainder (Sgr A-East) has a straight spectrum with index ~ -0.47 . Sgr A-West, with its small scale structure^{3,4}, could have a more complex spectrum than portrayed on Fig. 2 but no component could have a spectral index very much steeper than -0.5 .

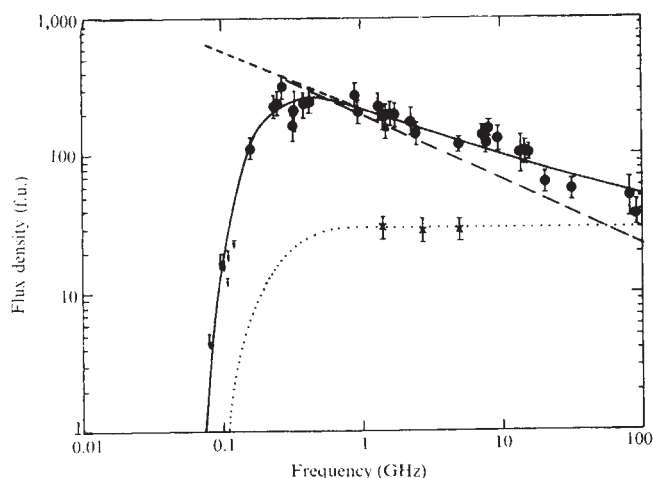


FIG. 2 Spectrum of Sgr A including the present value at 160 MHz. The dashed, dotted and full lines show the assumed spectra for Sgr A-East, Sgr A-West and the total flux density respectively. The falloff at low frequencies was derived from the extrapolated spectrum and the present measurement at 160 MHz. The points marked with crosses and triangles represent observations where Sgr A-East and Sgr A-West were resolved.

We now consider the decrease in flux at frequencies $\lesssim 400$ MHz. Neither a change in the electron energy spectrum nor a mixture of emission and absorption regions within the

source can account for the exponential decrease in flux. Instead it has been attributed to free-free absorption in the interstellar medium between Sgr A and the Earth^{9,10}. If we assume that the intrinsic spectrum of the non-thermal component continues unchanged to low frequencies, then the observed fall-off requires the optical depth at 160 MHz to be $\tau_{160} = 1.34$. The solid curve on Fig. 2 is the theoretical flux density curve which passes through our point at 160 MHz.

We can use the value of optical depth at 160 MHz to estimate the r.m.s. electron density $\langle n_e^2 \rangle^{1/2}$ in the interstellar medium if we make some assumptions about the electron temperature and filling factor. For a hot diffuse medium with $T_e = 5,000$ K distributed over the full 10 kpc to Sgr A we find $\langle n_e^2 \rangle^{1/2} \approx 1.6 \text{ cm}^{-3}$. For cold, dense clouds with $T_e \approx 50$ K occupying 2% of the line of sight we find $\langle n_e^2 \rangle^{1/2} \approx 0.6 \text{ cm}^{-3}$.

Lockman and Gordon¹¹ have observed weak recombination line emission at the position of Sgr A and at other locations within about 0.5 arc deg where no discrete source is visible. The range of T_e and n_e derived from these observations is in generally good agreement with our values above. In interpreting their observations, however, Lockman and Gordon¹¹ and Gordon⁴ have suggested an extremely cold, dense cloud model in which $n_e > 3 \text{ cm}^{-3}$ and $T_e \approx 20$ K. In such an extreme cloud, the absorption observed at 80 and 160 MHz would occur in a distance of only 3 pc. A cloud of this size, if located near the galactic centre, would subtend an angle of only 1 arc min. Our 80 and 160 MHz observations, however, suggest that the absorption is fairly uniform over an area of $\gtrsim 50$ (arc min)²; it is not patchy, as would be implied by this cloud model. We conclude that the extremely cold, dense cloud model is unlikely; to cover the source there must be one large cloud or many small clouds, each having a density lower than 3 cm^{-3} , a temperature higher than 20 K or both.

Observations of the radiation from supernova remnants show that absorption in the galactic plane is fairly common¹²⁻¹⁴, although not as strong as for Sgr A, and that the absorption is fairly smooth over the angular extent of the sources¹⁵.

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Upper limit to the flux of soft X rays from λ -Sco

DURING a rocket flight on May 26, 1971 which carried a number of large area thin window proportional counters, a soft X-ray source was observed by Bleeker *et al.*¹ in Scorpio. Photons in the energy range 0.37 keV to 1.9 keV were detected from a position error box of approximately 40 square degrees. This admittedly large error box contains the bright star λ -Sco and it was suggested that this object may be the source of the soft X-ray emission that was detected. It is a B1 V star and exhibits a varying radial velocity with a period of about 5.6 d. It has a visual magnitude of 1.62 and its distance is estimated as 100 pc (ref. 2).

Since the identification of an object such as λ -Sco as a soft X-ray emitter would be of considerable importance, we decided to analyse the data obtained with the MSSL low energy (0.5–1.5 keV) X-ray telescope while the Princeton University experiment was observing the same source at ultraviolet wavelengths. The X-ray instrument on Copernicus uses several grazing incidence paraboloidal reflectors with small gas proportional counters located at their foci. A brief description of this instrument has been given elsewhere³; a more complete description is to be published shortly. The 0.5 keV to 1.5 keV telescope system, used to observe λ -Sco, used a 10 arc min. field of view for the observations reported here.

The data were obtained during some 109 h of observation and are presented in Table 1*. They are split into blocks of about 12 h. No data block showed any signal above the background. For the five blocks of data starting on September 19, 1972, the shutter intended to obscure periodically the detector windows was in operation so that the particle background could be estimated accurately. The measured background counting rates were used to produce calculated background rates for the subsequent blocks when the shutter was inoperative.

Although Bleeker *et al.* were unable to estimate the spectral shape of the radiation which they detected, the absence of any signal in their higher energy channel suggests that the spectrum is steep. We have therefore assumed a thermal spectrum, with an electron temperature of 2×10^6 K, in deriving an upper limit to the X-ray luminosity of the source. We emphasise that there is no basis in our data for this assumption but that the results of Bleeker *et al.* show no flux above 1.9 keV. The thermal continuum expression used was of the form:

$$I(E) = K \exp(-E/kT_e) \exp(-N_H \sigma(E)) \quad (1)$$

where K is a normalisation constant, T_e is the electron temperature, N_H the interstellar hydrogen column density to the source² ($\sim 7 \times 10^{19}$ atoms cm^{-2}) and $\sigma(E)$ is the weighted average photoelectric absorption cross section for the material of the interstellar medium⁴. Using equation (1) and the values of the parameters quoted above, a 3σ upper limit was obtained to the source luminosity of $4.0 (\pm 1.0) \times 10^{32}$ erg s^{-1} for the energy range 0.5 to 1.5 keV.

Bleeker *et al.* quoted a value of 2×10^{33} erg s^{-1} so it would seem that the source detected by these workers was not the star λ -Sco. It is possible, as suggested by Bleeker *et al.*, that λ -Sco is a variable. Our observations cover between 50% and 80% of the radial velocity cycle, the uncertainty being due to the lack of an exact period for these variations. If the radial velocity variations were associated with any change in the X-ray flux, it is possible, though in our opinion unlikely, that the present observations might all have coincided with 'off' states of the X-ray emission.

It is possible that λ -Sco emits soft X rays in short bursts.

* See Table 1 on page 90

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If this were the case and if the outbursts were of the luminosity suggested by Bleeker *et al.* we would require about 10 min to see a signal of 3σ significance. The data were searched and no significant 10 minute blocks of data were found in 109 h of observation. In addition, a brief survey of this region by Hill *et al.*⁵, carried out about 1 h before the rocket flight of Bleeker *et al.*, failed to detect a significant signal from λ -Sco.

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Upper limit on the low energy interstellar cosmic-ray flux

DETERMINATIONS of the flux of low energy, non-solar cosmic rays extend at present down to 5 to 10 MeV for observations above the Earth¹. But the interstellar flux of cosmic rays below 1 GeV is highly uncertain because of solar modulation effects. The interstellar differential energy spectrum in the range 10 MeV to 1 GeV is usually deduced from theoretical models, by choosing more or less plausible interplanetary diffusion coefficients that will give a fit to the data. The state of sophistication of these theories is, however, still far from satisfactory, since predicted (low energy) values of the demodulated flux can vary by orders of magnitude among different authors, no experimental data on the interstellar flux having been available until now to help in selecting among different models.

In the present communication we wish to point out that there exists now an indirect experimental upper limit on this interstellar low energy flux, based on determinations of the state of ionization of the trace elements in the interstellar gas by the OAO-3 satellite Copernicus². A comparison of these results with those expected if the ionization were due to low-energy cosmic rays allows a direct upper limit to be placed on the number of ionizations per second that an interstellar hydrogen atom would suffer from cosmic rays³. This upper limit on the cosmic ray-induced ionization rate, ζ^{cr} , is

$$\zeta^{cr} < 4 \times 10^{-17} \text{ s}^{-1} \quad (1)$$

In order to obtain information on the flux from this limit, I use the definition

$$\zeta^{cr} = 4\pi \delta \int_{T_0}^{\infty} \phi(T) \sigma(T) dT \quad (2)$$

where $\phi(T)$ is the flux (particles $\text{cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (MeV per nucleon)⁻¹), δ is a factor allowing for secondary electrons and the effect of species with $Z > 1$, T is the cosmic-ray kinetic energy, and $\sigma(T)$ is given by the usual Bethe expression

$$\sigma(T) = 1.23 \times 10^{-20} Z^2 \beta^{-2} \cdot \{6.20 + \log [\beta^2/(1 - \beta^2)] - 0.43\beta^2\} \text{ cm}^2 \quad (3)$$

which is valid down to 0.3 MeV per nucleon.

In the case of cosmic ray protons, δ contains a factor 5/3 to allow for secondary electrons⁴, and a factor 2 to allow for species with $Z > 1$. Some of the expressions proposed for the interstellar proton flux are $\phi^p(T) = 1.2 \times 10^9 (T - 0.75 m_p c^2)^{-2.65}$ particles $\text{cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (MeV per nucleon)⁻¹ (see ref. 5) or for the proton density, $U(T) = 4\pi c \beta^{-1} \phi(T) = 9.14 \times 10^{-7} [(T + 1.5 m_p c^2)/m_p c^2]^{-2.75}$ particles m^{-3} (MeV per nucleon) (see ref. 6). If one computes with these spectra the ionization rate ζ^{cr} , using equations (2) and (3), it is found that the observational upper limit (1) is not violated, by an ample margin. This remains true even if we integrate below 0.3 MeV per nucleon, using the classical approximation cross section⁷. But the expressions quoted above for the spectrum are known to be inadequate for explaining the kinks and anomalies that appear below a few tens of MeV (refs 8, 9). To account for these, Fisk¹⁰ proposed that, after flattening in the usual manner below 1 GeV, the spectrum steepens again below ~ 85 MeV, in the form $\phi^p(T) = 4 \times 10^{11} T^{-5.5}$ particles $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (MeV per nucleon)⁻¹. This flux, in order not to violate expression (1), must cut off sharply at 30 MeV, or if the spectrum turns over with a slope $\propto [\sigma(T)]^{-1}$, then, the peak should be at $T > 30$ MeV. This is more stringent than the limit that would be obtained from the usual energy density arguments by a factor 2. Earlier, and also to explain the low energy kinks, Gloeckler and Jokipii¹¹ had proposed that the spectrum began rising again, below 40 MeV, as $\phi^p(T) = 6.2 \times 10^8 T^{-4.2}$ particles $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (MeV per nucleon)⁻¹. The observational upper limit here imposes a cutoff at energies $T \geq 20$ MeV. Finally, if one tried to extrapolate the high-energy flux as $\phi^p(T) T^{-2.65}$ down to MeV energies, and interpreted T as kinetic energy throughout (which may not be justified), the cutoff would have to come at $T \geq 50$ MeV. If one considers an arbitrary power law spectrum $\phi^p(T) = \phi_0 T^{-\alpha}$, taking $\sigma(T) \simeq \sigma_0 T^{-1}$ and assuming a cutoff at 1 MeV, one obtains the limit $\phi^p(1 \text{ MeV per nucleon}) < 5 \times 10^2$ particles $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (MeV per nucleon)⁻¹.

In the case of cosmic-ray electrons, I adopt 1/3 for the positron fraction of the total electron-positron flux, and try to interpret (1) as giving information on the electron flux. In this case $\delta = (5/3)(3/2) = 2.5$ (that is, I neglect proton, and $Z > 1$, contributions to ζ ; if this were not valid, the cutoffs derived below should be lower limits). Cummings *et al.*¹² find a good fit to the observed electron data above 10 MeV, using the diffusion-convection approximation and an assumed interstellar spectrum $\phi^e(T) = 3.16 \times 10^6 T^{-2.5}$ electrons $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ MeV}^{-1}$, which is a straight extrapolation of the flux observed above a few GeV. Condition (1) requires this flux to cutoff at $T \geq 6$ MeV, so the lower end of this assumed interstellar spectrum is dubious. In a different investigation, Cummings *et al.*¹³, from an analysis of the galactic non-thermal radio emission, derive a somewhat flatter 'nominal' spectrum $\phi^e(T) = 1.8 \times 10^4 T^{-1.75}$ electrons $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ MeV}^{-1}$ down to 80 MeV, and a possible 'high' spectrum, which below 80 MeV could be extrapolated (on the basis of their graphs) as $\phi^e(T) = 5 \times 10^6 T^{-1.75}$ electrons $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ MeV}^{-1}$. Proceeding as before, we find that these spectra cannot be extrapolated below 0.1 MeV for the 'nominal' spectrum and below 10 MeV for the 'high' spectrum. For an arbitrary power law spectrum $\phi^e(T) = \phi_0 T^{-\alpha}$, taking $\sigma(T) \simeq$ con-

stant $= 9.3 \times 10^{-20} \text{ cm}^{-2}$ above 1 MeV (this is the transition region for the electron cross section), one obtains, assuming a cutoff at 1 MeV, an upper limit $\phi^*(1 \text{ MeV per electron}) < 1.4 \times 10^5 (\alpha - 1) \text{ electrons m}^{-2} \text{ s}^{-1} \text{ sr}^{-1} (\text{MeV per electron})^{-1}$. The upper limit (1) thus leads to definite restrictions on the flux of low energy cosmic rays. It had been shown¹⁴ that, in order not to produce too much ^{11}B from spallation reactions, a limit could be set on the ionisation rate from protons in the range 5 to 30 MeV, $\zeta_{5-30} \lesssim 6 \times 10^{-17} \text{ s}^{-1}$. This restricted the range of low energy cosmic rays that had been postulated for heating the interstellar gas to the range $T < 5 \text{ MeV}$ (see ref. 15). Since my limit (1) of $\zeta < 4 \times 10^{-17} \text{ s}^{-1}$ is not restricted to an energy range, it effectively rules out cosmic rays with $T < 5 \text{ MeV}$ also. In fact, the cutoffs, when predicted, happened above this value.

I note that the limit (1) is, strictly speaking, valid within 100 pc from the Sun^{2,3}. In order to use this limit, or the cutoffs derived above, to derive conclusions about the diffuse radio, X-ray or γ -ray background, it should be kept in mind that integrations over a much longer path length are required, and a straightforward use of expression (1) may not be justified for these purposes. As far as solar modulation theories are concerned, the cutoffs derived above are probably more important in the electron case than in the proton case. This is because in the latter case the observed flux at 10 to 20 MeV is representative of a higher energy region in the interstellar spectrum⁵. But the necessity of cut-offs has been demonstrated only for the usual power law types of interstellar spectra discussed above. It may be that other types of spectra can be found to give agreement with the observed spectrum without violating condition (1). If these cutoffs are real, they may provide some information on the sources of cosmic rays. For instance, under the assumption of an isotropic, steady-state injection (such as pulsars and white dwarfs), if we assume that the low-energy particles stream with the Alfvén velocity of the ionized component of the interstellar gas, then since the lifetime of a 20 MeV proton is $\tau \simeq 1.5 \times 10^6 \text{ yr}$, the distance between sources must be $\gtrsim 30 \text{ pc}$. Or, if low energy cosmic rays are injected by supernova events, some information on the frequency or spacing of these events may also be gained from the fact that the surviving cosmic rays must have $\tau \simeq 1.5 \times 10^6 \text{ yr}$. Alternatively, the necessity of a cutoff may not reflect some characteristic of the spatial and temporal distribution of the sources, but rather may be inherent to the acceleration mechanism itself.

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Magnetisation of comets

THE presence of magnetic fields in the plasma tails of comets is suggested by the rayed structures and helical features observed in them¹. According to the present picture of the solar wind-comet interaction (see, for example, ref. 2) it is, however, not at all clear how the interplanetary magnetic field could mix with the cometary plasma in the tail because the solar wind and the associated interplanetary field is separated from the cometary plasma by a contact surface.

Although it has been suggested that the contact surface may be liable to various instabilities such that the interplanetary magnetic field may penetrate it and establish contact with the plasma in the tail³ the viability of the process has not yet been demonstrated by any detailed analysis. It seems therefore legitimate to question if significant magnetic fields can be generated within the coma and sustained there for sufficiently long periods of time.

There are good reasons to believe that the magnetic fields of celestial bodies derive from the hydromagnetic conversion of kinetic energy into magnetic energy in their cores, although the details of the conversion mechanism are less clear. One such mechanism is provided by the well known 'magneto-hydrodynamic dynamo' (see, for example, ref. 4). An alternative and less speculative mechanism is the 'poloidal field amplification' mechanism which is founded on phenomena observed in laboratory experiments⁵ and which has been recently used for a theory of the Earth's magnetic field (H. A. and L. Lindberg, paper presented at the Geophysical and Geochemical Conference in Houston, Texas; January, 1973). Independent of which mechanism is preferred, however, the general requirements for a fluid body to be magnetised are that its volume and electrical conductivity are large enough, that there is sufficient internal kinetic energy to be converted to magnetic energy, and that there is a degree of ordering of the internal (turbulent) velocity field such as would be provided by rotation. The magnetic field H generated should be of the order

$$H = (4\pi\rho v^2)^{1/2} \quad (1)$$

where ρ is the density and v is the velocity of relative motion ('turbulent' velocity).

Clearly gas streaming out radially from a central nucleus cannot produce such magnetic field amplification, but if the nuclear region ($r \lesssim 10^4 \text{ km}$) consists of a supplementary distributed source for the coma gases as has been suggested recently by several authors^{6,7} a turbulent flow with maximum velocities of the order of thermal velocity ($\approx 1 \text{ km s}^{-1}$; ref. 7) may be expected in this region, with the necessary ordering in the turbulent velocity field provided by the rotation of the nuclear region. Taking $(\rho) \approx 5 \times 10^{-16} \text{ g cm}^{-3}$ as a typical value in $r \lesssim 10^4 \text{ km}$ (ref. 7) and putting $v = 1 \text{ km s}^{-1}$, we obtain from equation (1) $H \approx 0.01\text{G}$, which is a significant field. Even if the turbulent velocity, v , were an order of magnitude smaller, H would still amount to about 100 γ .

It is necessary to check if this magnetic field can be retained for a sufficiently long time. The time of decay τ , of the magnetic field is given by

$$\tau = 4\pi L^2 \sigma \quad (2)$$

where σ is the electrical conductivity (e.m.u.) and L is the scale length of the turbulent eddies.

In estimating the electrical conductivity of a partially ionised gas, as is the one under consideration, it is important to check if the lifetime of the electrons is sufficiently long, for otherwise the conductivity will be provided mainly by the much less mobile positive and negative ions (when it will be decreased essentially by a factor $\sim (m_i/m_e)^{1/2} \sim 100$). Although comets contain highly electronegative gases like OH the lifetime against electron attachment at the relevant densities ($n \approx 5 \times 10^7 \text{ cm}^{-3}$) is more than 10^7 s (ref. 8), about two or three orders of magnitude larger than the ionisation time scales of some of the dominant coma species (for example, CO)⁹. Consequently the appropriate conductivity is the electron conductivity, which is given by¹⁰

$$\sigma = \frac{4 \times 10^{-19} (n_e/n)}{T^{1/2} \langle Q \rangle} \text{ e.m.u.} \quad (3)$$

where $\langle Q \rangle$ is the effective collision cross section for electrons and neutral atoms and T is the electron temperature. Without a better knowledge of both the chemical composition and the photoionization processes in the inner coma it is not possible to estimate n_i/n in $r \lesssim 10^4 \text{ km}$ with any degree of certainty. If, however, we make the not unreasonable assumptions that $n_{\text{CO}}/n \approx 0.1$ and that the degree of ionisation of CO in $r \lesssim 10^4 \text{ km} \approx 0.5$, then taking $T \approx 1,000 \text{ K}$ and $\langle Q \rangle \approx 10^{-15} \text{ cm}^2$, we get $\sigma \approx 5 \times 10^{-7} \text{ e.m.u.}$ Once again there is an uncertainty about the value to be ascribed to L , but if we take $L \approx 10^3 \text{ km}$ $\tau \approx 2,000 \text{ yr}$ and if we take $L \approx 10^2 \text{ km}$ $\tau \approx 20 \text{ yr}$. Even if our smallest estimate of τ is too large by two orders of magnitude we still obtain $\tau = 0.2 \text{ yr}$, which is yet of the order of the periods of coma and tail activity of a typical comet.

It therefore seems to us that a comet, when sufficiently close to the Sun, may be able to generate an appreciable internal magnetic field and that the magnetic fields observed in the tail may then result from processes analogous to those producing the Earth's magnetotail.

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Geochemical evidence for an east-dipping Appalachian subduction zone in Newfoundland

THE concept of plate tectonics explains the present behaviour of the Earth better than any rival hypothesis. Modern plate motion can be observed and measured, but unfortunately much of the evidence is ephemeral. Spreading ridges, trenches, oceanic magnetic lineaments, zones of seismic activity dipping under orogenic belts, and areas of anomalous heat flow are all destroyed or decay as the plate regime evolves. Applicability of the plate tectonic concept to the ancient past must therefore be determined by more indirect geological observations which are often open to more than one interpretation. For example, it can be inferred that melanges should develop in trenches associated with subduction¹, but not all melanges originate in this way and there is presently no easy way of typefying melanges. Thus, if the great variety of presently observed plate interactions existed in the past, geologic data may not be subtle enough to distinguish them and the record may be blurred beyond reasonable interpretation.

Given the limitations of geological data, it seems most reasonable to use the gross regional information rather than the details of local areas as a first basis for evaluating ancient plate tectonics. It also seems more reasonable to accept, at least temporarily, the simplest model suggested by these regional data rather than construct complex models to explain variations in local details.

There are several gross geochemical trends observed across presently active subduction zones. The potassium content of igneous rocks varies systematically across both island arcs²⁻⁴ and Cordilleran mountain chains⁵⁻⁷: with increasing distance from the trench, the potassium content of igneous rocks increases. There is also a systematic zonation of mineral deposits across several active orogenic zones with progressively more lithophile elements being concentrated away from the trench above the subduction zones⁸⁻¹⁰. Although these geochemical trends have not been clearly observed across all presently active subduction zones, there are no substantiated cases where a reverse zonation has been established.

Other geological features which may be systematically related to subduction zones include paired metamorphic belts¹¹, ophiolite emplacement¹², melange zones¹, and piles of calc-alkaline volcanic rocks. But these features by themselves are not yet unambiguous indicators of the reality or nature of plate movements in the Palaeozoic, and are especially difficult to interpret for directions of dip of paleo-subduction zones.

The various plate tectonic models that have been proposed for the Appalachian-Caledonian mountain system are summarized in Fig. 1. Most of the models presuppose plate tectonics and support the assumption with local geological data of varying reliability and significance. It is noteworthy that all the models based on empirical geochemical data are consistent, and all indicate an ancient subduction zone dipping away from the proto-North American continent in a present easterly or southeasterly direction. The models for Great Britain and Norway (ref. 15 and G. H. Gale and F. M. Vokes, unpublished) are based on the chemical composition of igneous rocks and/or the general zonation of mineral deposits. These geochemical patterns strongly resemble those across presently active subduction zones, and they represent the most direct non-palaeomagnetic evidence for Palaeozoic plate movements.

Our data suggesting an easterly dipping Appalachian subduction zone in Newfoundland are derived from a study of both the geochemistry of igneous rocks and the present distribution of mineral deposits. They are supported by

other geological data.

More than 1,200 samples of granitic rocks from thirty-three plutons in Newfoundland have recently analysed in detail²⁶. These analyses show that, irrespective of geological

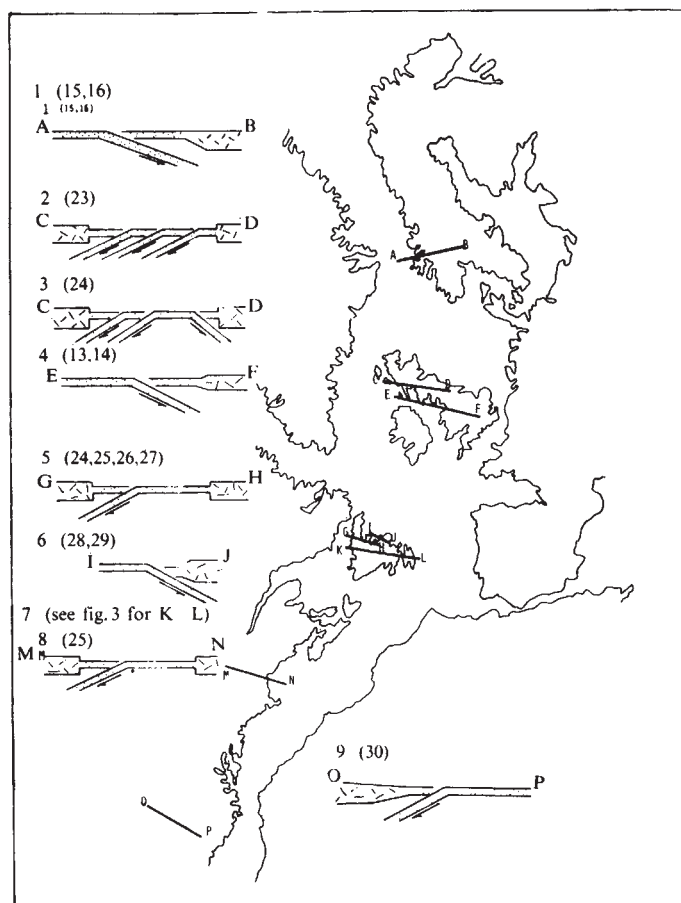


FIG. 1 Summary of plate tectonic models suggested for different sections of the Appalachians-Caledonides. Numbers in brackets next to each model refer to references. Stippled areas represent oceanic-island arc crust and lined areas represent continental crust. Map drawn from reconstruction by Bullard, *et al.*³⁰. Note that only models 1, 4 and 6 are supported by chemical data. *Unpublished results of G. H. Gale and F. M. Vokes.

age, there is a definite eastwards increase in the average potassium content of the plutons in the Central and Gander zones of eastern Newfoundland (Fig. 2). The plutons of the eastern Avalon zone do not seem to follow this trend and are therefore anomalous. This distribution of potassium is similar to that described from western North American and explained by derivation of magma from an east-dipping subduction zone⁵⁻⁷. The Avalon anomaly is comparable with the disjunct observed by Lipman, *et al.*⁷ and interpreted by them as indicating a second subduction zone. Further data may substantiate such an interpretation in Newfoundland, but our present data allow us only to make a very tentative postulation of a second subduction zone responsible for the Avalon plutonic rocks. Preliminary inspection of our data suggests that the main subduction zone dipped at a rather steep angle, perhaps as much as 60°, eastwards. A steep dip would also explain the relative narrowness of the Appalachians as compared to the Cordillera.

The zonation of Newfoundland mineral deposits¹⁷ also indicates an eastward dipping subduction zone (Fig. 3). The observed distribution is like that described by Sillitoe^{8,9} in western North and South America, Mitchell and Garson¹⁰ for the southwest Pacific and G. H. Gale and F. M. Vokes (unpublished) for Norway. Several other features of New-

foundland geology are also satisfactorily explained by a long lived east-dipping subduction zone.

The ophiolites of west Newfoundland and the Burlington Peninsula represent obducted oceanic lithosphere^{25,27}. Their westward emplacement is most simply explained by an over-

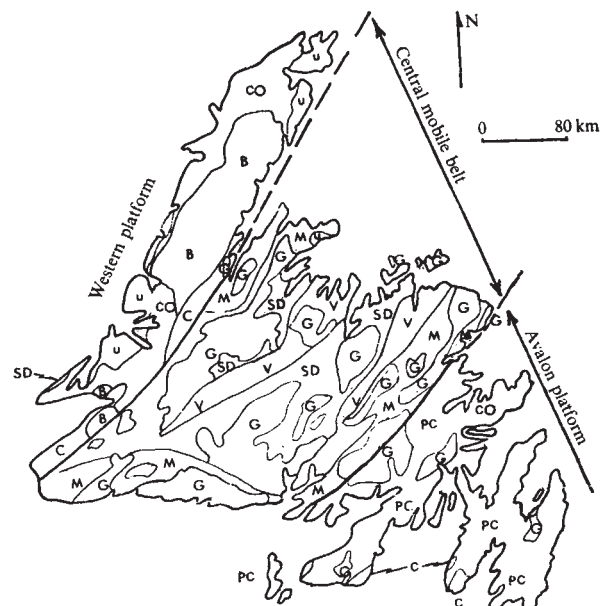


FIG. 2 Simplified geological map of the island of Newfoundland. G, Granites; u, ophiolite sequences; C, Carboniferous sediments; SD, Silurian and Devonian subaerial sandstones, conglomerates and shales; V, Ordovician pillow lavas and deep set sediments; CO, Cambrian and Ordovician sandstones, limestones and shales; M, Pre-Ordovician schists and gneisses; PC, Precambrian sediments and lavas; B, Grenville basement gneisses, granites and anorthosites.

thrusting of oceanic lithosphere onto the proto-North American continent above an east-dipping subduction zone, accompanied by an imbricate stacking up of the continental margin sediments under the ophiolites, all of which now form the west coast allochthons.

TABLE 1 Points of geological comparison between eastern and western Newfoundland

Western	Eastern
(1) Grenville Basement.	None definitely known, except possibly in the Gander Lake zone.
(2) Hadrynian plateau basalt volcanism.	Hadrynian acid ignimbrite volcanism.
(3) Fleur-de-Lys metamorphic zone.	Gander Lake metamorphic zone.
(4) Cambro-Ordovician shelf limestones.	Cambro-Ordovician Mn-Fe oxides.
(5) Ophiolites obducted onto western platform.	Ophiolites not obducted, just deformed and intrusive ultramafics.
(6) No post-Grenville granitoid rocks present.	Granitoid rocks possibly ranging from Hadrynian to Devonian in age.

The calc-alkaline piles of early and middle Ordovician volcanic rock with associated sediment in Notre Dame Bay would represent the volcanic island arcs associated with such eastward subduction.

The Gander Lake metamorphic belt would result from metamorphism along a continental margin above the subduction zone. This zone passes eastwards into the Avalon Platform continental crust, which was then a Basin and

Range type regime marked by strong faulting and bimodal basalt-ignimbrite eruptions. This Cordilleran type mountain chain might have been the long lived New Brunswick geanticline of Schuchert¹⁸. Its existence might also explain the inferred long age span of granites of the Gander Lake zone¹⁹.

The western Fleur-de-Lys metamorphic and plutonic zone would be explained according to the model of an east-dipping subduction zone as being the result of the overriding effect of oceanic lithosphere, that is subduction under oceanic lithosphere, with a resulting sinking, deformation and melting at the base of the continental crust.

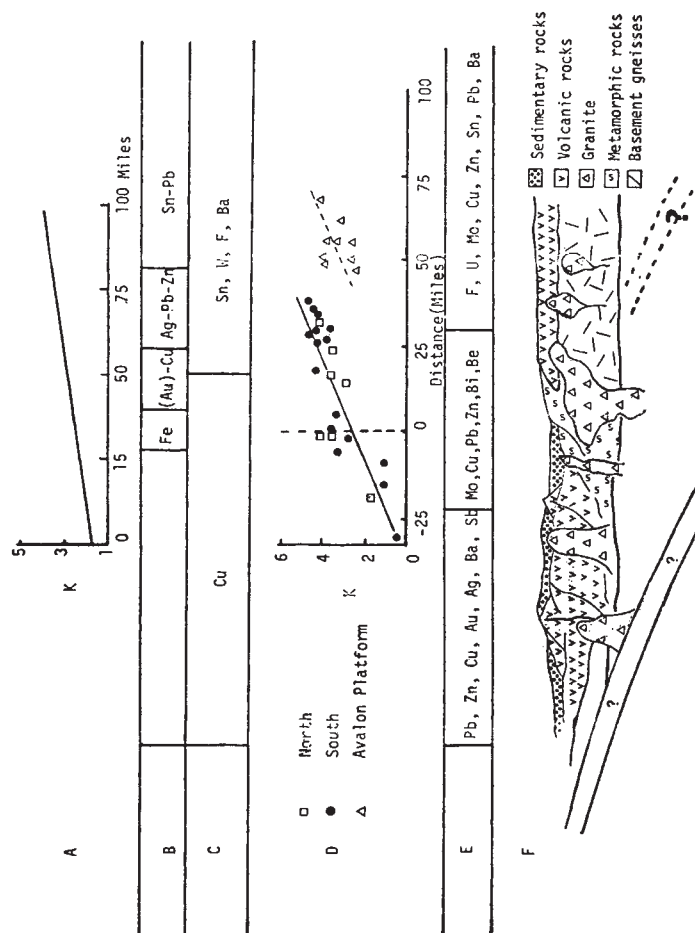


FIG. 3 Summary of geochemical and metallogenic evidence supporting an eastward-dipping Appalachian subduction zone in Newfoundland, as compared to analogous areas elsewhere. A, Variation in K across the Central Sierra Nevada Batholith (after Bateman and Dodge⁵). B, Metal zoning of western North and South America (after Sillitoe⁸). C, Metal zoning across Circum-Pacific island arcs (after Mitchell and Garson¹⁰). D, Variations in K across eastern Newfoundland. The K data points are means for individual plutons (>1,200 analyses in total), the positions of which are plotted as distance of pluton centers from the western margin of the Gander Lake metamorphic belt, which is taken as the eastern Appalachian continental margin. E, Metal zoning in eastern Newfoundland. F, Schematic plate tectonic interpretation of the data described above.

continental margins²¹ we emphasise that there is a fundamental asymmetry evident on a finer scale (Table 1, Fig. 2). It can be explained as the result of an east-dipping subduction zone. The most striking aspect of this asymmetry is that there are no post-Grenville granitoid rocks on the western platform, that is, they seem to terminate abruptly at a line approximately along the Cabot fault. This line is taken as the westward limit of subduction.

The inferred existence of a subduction zone active for more than 250 m.y. suggests that the proto-Atlantic was a rather large ocean basin.

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Although the gross geological symmetry of Newfoundland²⁰ can be explained by the bringing together of two

New approach to surface seawater palaeotemperatures using $^{18}\text{O}/^{16}\text{O}$ ratios in silica of diatom frustules

THE oxygen isotopic composition of calcium carbonate and silica deposited by organisms in water depends on the temperature and the isotopic composition of the water¹.

As deep seawater temperatures probably did not vary during the Pleistocene, the variations of the oxygen isotopic composition of the sea during this period can be followed by analysis of the $^{18}\text{O}/^{16}\text{O}$ ratio in the tests of deep benthic foraminifera². Shallow pelagic foraminifera grew in waters where significant temperature variations seem to have occurred in glacial periods. Yet the $^{18}\text{O}/^{16}\text{O}$ ratio in their carbonate tests does not show such variations. An explanation of this phenomenon lies in the ability of pelagic foraminifera to adjust the depth at which they live so as to minimise the effect of temperature variations on their biology^{2,3}.

To be able to follow oceanic surface palaeotemperatures by the variations of the $^{18}\text{O}/^{16}\text{O}$ ratio in a compound, it is necessary to be sure that the site of formation was really near the surface. Diatoms, most of which form skeletons of silica, grow in the upper 40 m because they require light for photosynthesis. Consequently, the $^{18}\text{O}/^{16}\text{O}$ ratio of their skeletons is dependent on the $\text{H}_2^{18}\text{O}/\text{H}_2^{16}\text{O}$ ratio and on the temperature of the surface water. Since the $\text{H}_2^{18}\text{O}/\text{H}_2^{16}\text{O}$ variations within a water column are very small, the $^{18}\text{O}/^{16}\text{O}$ ratio of benthic foraminifera of the same area can be used as an index of $\text{H}_2^{18}\text{O}/\text{H}_2^{16}\text{O}$ variations. Then, by comparing the variation of the $^{18}\text{O}/^{16}\text{O}$ ratio in silica from diatoms from different horizons of a core with that of benthic foraminiferal carbonates of the same horizons, it should be possible to calculate the surface water variations of $^{18}\text{O}/^{16}\text{O}$ ratios and temperatures during the past.

To test whether biogenous silica can be used for this purpose, it was necessary to check that it is formed in isotopic equilibrium with water, and to find the temperature relationship for $^{18}\text{O}/^{16}\text{O}$ fractionation between silica and water. For this study I analysed recent or living specimens, formed under known conditions of temperature and isotopic composition of the water.

Oxygen extraction from silica was carried out using BrF_5 by a method modified⁴ from that described by Clayton and Mayeda⁵. Detrital minerals, organic matter and the hydration water of the biogenous silica were separated from the specimens analysed. Removal of all of the detrital minerals

requires many filtrations and ultrasonic washings, and much settling and decanting⁴. Slow solution of carbonates with 0.1 M HCl is possible without oxygen isotopic exchange between the silica and the water. Organic matter is more difficult to destroy without interfering with the oxygen of the silica. But, since the organic oxygen has an isotopic composition approximately 15‰ lower, and is present as the major part of the total oxygen in recent samples, such a separation is necessary. We attribute the variations of oxygen isotopic composition seen by Mopper and Garlick⁶ on unwashed radiolaria to the slow degradation of the organic matter⁴. It is possible, using slow oxidation by NaOCl, to destroy more than 99% of the organic matter, even in fresh specimens⁷. A more serious contamination problem results from the physical nature of biogenous silica. This silica is formed in a low crystalline, highly hydrated state. In a few million years, the crystallinity increases, with progressive loss of water. This process can be accelerated by heat: The water trapped in the pores of the structure, which is in isotopic equilibrium with the environment, is volatilized by heating above 100° C in vacuum, but without apparent exchange with the oxygen of the silica. The remainder of the water (approximately 2 to 4% by weight of the silica) is partly chemically bound, in the hydroxyl form, to the silicon atoms. Heating at about 1,000° C in vacuum removes this water⁸ by increasing the crystallinity towards tridymite, cristoballite, or even quartz. To see the effect of this dehydration procedure on the isotopic composition of biogenous silica, the $^{18}\text{O}/^{16}\text{O}$ ratio of a Miocene diatomaceous earth from Cantal (France) was determined, after heating in vacuum for 1 h different samples of the same weight at different temperatures (Fig. 1). The isotopic composition is changed in a non-reproducible manner by heating between 500° C and 600° C. As most of the water is ejected in the first few minutes of heating, 1 h of treatment permits a reproducible dehydration. To ensure that the water is volatilised at constant temperature, we use a modified furnace, in which the sample reaches the plateau temperature in a few seconds. The scatter of the low temperature data indicates that rapid but incomplete isotope exchange occurs during dehydration under these conditions, partly because of interactions with residual organic matter lost at these temperatures⁹. On the other hand, the isotopic composition of specimens dehydrated at temperatures greater than 800° C is nearly constant and about 1‰ higher than that of specimens heated at temperatures below 500° C. There is only weak isotopic fractionation during exchange be-

TABLE 1 Samples and conditions of growth

Sample and origin	Water temperature °C	$\delta^{18}\text{O}$ of the water ‰ relative to snow	$\delta^{18}\text{O}$ of the samples ‰ relative to snow	$\Delta^{18}\text{O}_{\text{SiO}_2-\text{H}_2\text{O}}$ (‰) $= \delta^{18}\text{O}_{\text{SiO}_2} - \delta^{18}\text{O}_{\text{H}_2\text{O}}$	$\Delta^{18}\text{O}_{\text{SiO}_2-\text{H}_2\text{O}}$ (‰)
Sponges					
Indian Ocean (Kerguelen)	4 ± 1	-0.25 ± 0.25	40.20 40.45	40.45 40.70	40.6
Mediterranean Sea (Banyuls)	15.5 ± 2	+1.3 ± 0.2	39.30 39.50	38.00 38.20	38.1
Atlantic Ocean 37°48'N, 25°53'W (dredged at 700 m)	9 ± 1	+0.1 ± 0.1	38.20 38.30 40.20	38.10 38.20 40.10	38.15
36°47'N, 33°13'W (dredged at 2,000 m)	4.3 ± 0.3	+0.1 ± 0.1	40.35 40.40 40.30	40.25 40.30 40.20	40.2
Bahamas	27 ± 2	+0.8 ± 0.3	36.40	35.60	35.6
English Channel (Roscoff)	12 ± 2	0 ± 0.2	37.90 38.20	37.90 38.20	38.15
Diatoms			31.40	38.90	
Lake Pavin (France)	9 ± 3	-7.5 ± 0.1	31.60 31.90 32.0	39.10 39.40 39.50	39.2
Lake Myvatn (Iceland)	4.5 ± 2	-8 ± 1	32.15 32.45	40.15 40.45	40.3
Gulf of California 27°47'N, 111°25'W	25 ± 2	+0.6 ± 0.5	35.80	35.20	35.2

tween water and silica at these temperatures. So, if exchange occurs between silica and water during the dehydration process, the isotopic composition of the sample would remain constant. As the corresponding weight loss is only about 3%, the 1‰ ^{18}O enrichment is consistent with a water loss of isotopic composition approximately -40‰ compared to the silica. To these freshwater diatomites, $\delta^{18}\text{O} \sim 32\text{‰}$ relative to Standard Mean Ocean Water (SMOW), would correspond a water of isotopic composition -8‰ relative to SMOW, a value consistent with present freshwater composition in the sampling area. The 1‰ change in isotopic composition then can be interpreted as corresponding to a dehydration sufficiently fast to preclude isotopic exchange between water and silica. Then the isotopic composition of the silica dried above 800°C represents that of the silica as formed originally. This method has the advantage of producing crystalline, marginally hygroscopic, stable silica. The dispersion of measurements of fossil specimen isotopic composition was found to be less than $\pm 0.1\text{‰}$.

In order that silica formed under various regimens of water temperature and isotopic composition might be studied, newly deposited diatom frustules and siliceous spicules from living sponges were analysed. Preliminary results of the measurements are reported in Table 1, including the temperature and the $\delta^{18}\text{O}$ of the water during skeleton formation (as defined in ref. 9). Temperature during skeleton formation is presented as a function of the fractionation factor in Fig. 2. The regression line, based on the best defined point: $5 = 5 \pm 0.3^\circ\text{C}$; $\Delta(\text{SiO}_2\text{-H}_2\text{O}) = 40.0 \pm 0.2\text{‰}$ is described by the equation: $t(^{\circ}\text{C}) = 5 - 4.1 (\delta\text{SiO}_2 - \delta\text{H}_2\text{O} - 40)$. The 1σ uncertainty of the slope is ± 0.4 .

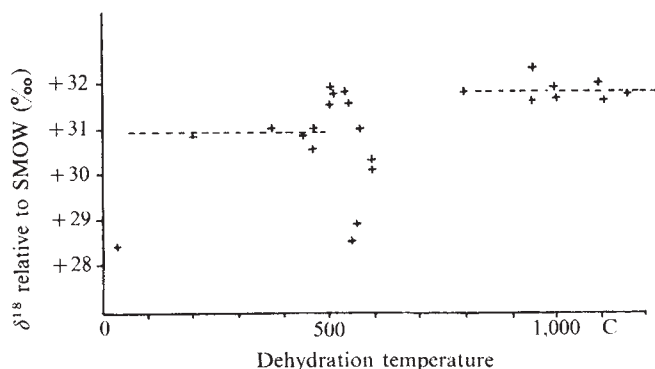


FIG. 1 Isotopic composition of the diatomite from LaBade (Cantal, France) after dehydration at different temperatures.

For palaeotemperature estimation, this uncertainty should be reduced by further analyses of warmer water specimens. But, within the present precision, the isotopic fractionation between water and either sponge spicule silica or diatom frustule silica is identical. There is then no indication of biogenic fractionation. In addition, data from freshwater samples from Lake Pavin and Lake Myvatn fell perfectly on the regression line, although these specimens were formed in water of $\delta^{18}\text{O}$ considerably different from the SMOW. The non-dependence of the isotopic enrichment of siliceous skeletons on the isotopic composition of the water in which they were formed is considered to indicate formation under conditions of isotopic equilibrium. This hypothesis is strengthened by the results of Clayton, O'Neil and Mayeda¹⁰ on the quartz water fractionation under pressure at temperatures over 200°C . We have shown⁴ that an extrapolation to room temperature of these results is valid, and gives a perfect agreement with our analysis.

To use $^{18}\text{O}/^{16}\text{O}$ in diatom silica for a Pleistocene palaeotemperature record, we must be sure this silica preserves its original isotopic composition on a scale of millions of years.

Some analyses of Miocene diatoms from fresh or marine waters exposed for a few million years to ground waters indicate no exchange in that period of time with the local ground waters⁴. But the long term variations, as observed by Degens and Epstein¹¹, restrict the application of this method to samples younger than some 10^7 yr.

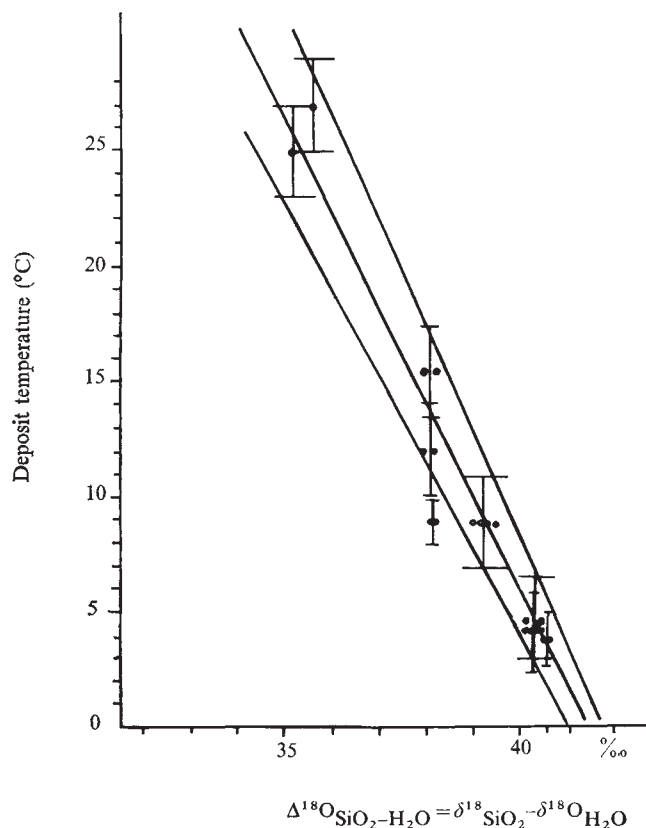


FIG. 2 Isotopic fractionation between water and silica from either diatoms or sponges, as a function of the temperature of formation. Centre line is regression line; outer lines define 1σ envelope. ○, Diatoms (frustules); ●, sponges (spicules).

In conclusion, the relationship found for the silica-water isotopic fractionation ($t = 5 - 4.1 (\delta\text{SiO}_2 - \delta\text{H}_2\text{O} - 40)$) is very similar to the known relationship for carbonates ($t = 16.9 - 4.2 (\delta'\text{CaCO}_3 - \delta'\text{H}_2\text{O})$). The slopes of these regression lines are too close to allow, through comparison of their $^{18}\text{O}/^{16}\text{O}$ ratios, the calculation of the temperature of the water in which siliceous and carbonate skeletons were formed simultaneously. But these results indicate that the measurement in sediments of $\delta^{18}\text{O}$ in frustules of diatoms and in associated tests of benthic foraminifera may be used to follow the variations of surface water temperature during the Pleistocene.

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Glacial advance relative to volcanic activity since 1500 AD

THERE may be a connection between the periodicity of glacial advance and the occurrence of volcanic eruptions. Lamb¹ has presented a graph which shows a similar curve for Arctic sea ice off Iceland and great Icelandic volcanic eruptions since 870 AD and Bray² found the number of maximum advances of alpine glaciers since 1680 AD is significantly related ($\chi^2 = 7.8$, $P < 0.01$) to intervals with a volcanic dust veil index of 1,000 or greater. The volcanic dust estimates have now been extended to 1500 AD (ref. 1) and in this paper I analyse the relationship between these estimates and records of maximum glacial advance from 1500 AD to the present.

Glacial activity was assessed by a compilation of maximum advance dates, defined as all advances which reached the terminal moraine or its near vicinity. Only dates based on historical observations or on distortions in the growth rings of trees standing at the time of glacial advances were selected, in order to ensure a maximum accuracy. A total of 128 dates were compiled from summary studies of which 16 dates were from North American trees²⁻⁵ and 112 were from European historical observations^{2,6-8}. The North American and European dates were synchronous.

The compiled maximum glacial advance dates are shown in Table 1 at 20 yr intervals together with Lamb's estimate of the amount of volcanic dust in the atmosphere of the Northern Hemisphere (from Table 7a of ref. 1). Both the glacial advance and volcanic dust data have a series of similar peaks during the 17th, 18th and 19th centuries, with lower values in the 16th and 20th centuries. There is a significant positive correlation ($r = +0.43$, $P < 0.05$) between the glacial and volcanic activity data in Table 1 which supports the results of the previous study². The basic data summarised in Table 1 show a tendency for glacial advance to follow major volcanic eruptions which may reflect the lag between the time of ice buildup and its maximum advance (as noted below).

A mechanism for the apparent relationship between glacial advance and volcanic eruption has been suggested²: After a major volcanic eruption, there is a global temperature decline from atmospheric dust for up to 3 or 4 yr or more². During this decline, there are colder springs and summers¹ and as a result, some precipitation which usually falls as warm storms over the icefields (thus promoting ablation), falls instead as snow. These snowfalls can increase the summer albedo of the icefield up to four times (albedo old ice ~ 20%, new snow ~ 80%) and together with a decrease in the number of warm storms is a major influence² in producing a strong positive ice balance and a consequent glacial advance.

This proposed sequence of major volcanic eruptions followed by several years of cold summers and then by glacial advance is supported by historic and phenologic data. Of

TABLE 1 Maximum glacial advances and atmospheric volcanic dust in the Northern Hemisphere 1500 to 1960 AD

Year AD	No. of maximum glacial advances	Dust Veil Index (DVI)
1500-19	0	650
1520-39	0	150
1540-59	1	1,250
1560-79	1	0
1580-99	3	1,090
1600-19	9	1,810
1620-39	5	825
1640-59	8	1,475
1660-79	3	1,675
1680-99	6	1,480
1700-19	12	1,220
1720-39	1	815
1740-59	9	1,280
1760-79	6	550
1780-99	4	1,940
1800-19	16	2,545
1820-39	12	3,180
1840-59	24	1,140
1860-79	2	1,120
1880-99	5	1,690
1900-19	1	770
1920-39	0	0
1940-59	0	400

the 102 late wine harvests which occurred on or after October 10 from 1500 AD to 1880 AD in France (data converted to a national basis from Appendix 12 of ref. 6), 25 occurred in the 59 yr with volcanic eruptions with DVI (Dust Veil Index) of 300 or greater ($\chi^2 = 6.3$, $P < .01$). A significant relationship ($\chi^2 = 27.0$, $P < 0.001$) with volcanic eruption was also determined for the 128 maximum glacial advance dates of which 111 occurred within 24 yr after the 23 volcanic eruptions with a DVI of 1,000 or greater from 1500 to 1970 (Table 2). The distribution of the lag between volcanic eruption and glacial advance shown in Table 2 is in agreement with studies³ of the interval between ice buildup in the icefield and subsequent maximum glacial advance.

Sequences of volcanic eruptions followed by cold summers and then glacial advance are shown in Table 3 which summarises: (1) all the intervals of 10 yr or less with volcanic eruptions with a total DVI of at least 1,000 (from Appendix I, ref. 1); (2) all years of cold summers in England (ref. 1, page 494) and France (ref. 6, pages 56, 58, 60) and years of famine and food shortage due to cold summers in western Europe and Japan (ref. 2, Table VII; ref. 1, page 494; ref. 6, pages 66-68, 92); and (3) the 128 glacial advance dates. The most notable sequences in Table 3 are those following the two largest volcanic eruptions, 1815 and 1835. The 1815 eruption was the peak of a series of eruptions from 1811 to 1817. Cold springs and summers, late wine harvests and food shortages occurred from 1812 to 1817 and included the "year without a summer" in 1816. A major glacial expansion began in 1814, maximum advances were reached from 1817 to 1824 and ice retreat began in 1819.

TABLE 2 Maximum glacial advances in the Northern Hemisphere in relation to global volcanic eruptions $\geq 1,000$ DVI, 1500-1970 AD

No. of years following eruption $\geq 1,000$ DVI	No. of maximum glacial advances Expected	Observed	χ^2
0-4	29.3	37	2.0
5-9	25.9	41	8.8
10-14	17.7	23	1.7
15-24	20.7	10	5.5
25-44	19.8	8	7.0
45-75	14.3	9	2.0
			27.0

TABLE 3 Sequences of volcanic eruption, cold summers and maximum glacial advance, 1500–1970 AD

Volcanic eruptions, Year AD	global DVI	Cold summers, late harvests, food shortages, Europe and or Japan Year AD	Maximum glacial advances, Northern Hemisphere Year AD	No.
(1912	500)	1912		
1895*, 1902	2,300	1903	1898–1910	2
1883*, 85, 86, 88	3,150		1883–94	3
1875*	1,000	1879	1881	1
(1868–70	1,000)†	1866–69		
1856, 61	1,500		1860–71	2
1845*, 46	1,800	1840s	1840–55¶	24
1835	4,000	1833–39		
1821, 22	1,100			
1811–1815*, 17	4,800	1812–17	1817–24¶	26
1795*, 96, 98, 99	2,200		1807–09	2
1779, 83*, 86	4,050	1782–87	1786–89	3
1763, 66*, 68	4,450	1760s	1771–80	5
1752*, 54, 55	2,500	1755	1756–60	3
(1730–1736	700)	1740–42	1736–43	9
1717, 21, 24	1,600	1725		
1707, 12	1,600	1709, 1711–17	1712–20¶	10
1693, 94	1,800†	1692–98	1694–1700	6
1680*	1,000			
1673*	1,000	1672–75	1676–80¶	5
1660*, 64	2,900			
(1646, 50	800)	1646–50	1653	1
1636, 38, 40, 41*, 46	2,900	1639–43	1640–44¶	7
1625, 31	1,400	1627–33	1628–30¶	5
1614*	1,000	1618–21		
1601*, 06	1,550		1610	2
1593*, 97	1,300	1594–97	1589–1601¶	10
1586*	1,000	1586–87		
1553*–54*	1,000	1555	1576	1
		1527–29	1546	1
1500*	1,000			

Years which had eruptions > 1,000 DVI; †DVI could be as high as 3,000 to 3,500; ‡DVI estimated from temperature anomalies; ¶underlined glacial phases are those emphasized by Ladurie for western Europe (pp 195, 211 ref. 5).

The 1835 volcanic eruption, together with subsequent eruptions in 1845 and 1846, was followed by cold springs and summers, late wine harvests and food shortages in 1833–39 and the 1840s with a major glacial advance period occurring from 1840 to 1855. All the glacial expansion phases with five or more advances were preceded by major volcanic eruptions except that of 1736–43 which was preceded by a period of cold summers, food shortages and severe famine in 1740–42, but by only a minor volcanic eruption of 700 DVI from 1730–36. The 1527–29 food shortage period was apparently not related to a preceding volcanic eruption.

The role of global temperature (perhaps controlled, in part, by solar activity²) in the proposed volcanic eruption-glacial advance sequence may be to determine the number and magnitude of glacial advances. This may be shown by the relatively lower number of advances following volcanic eruptions during the generally warmer period¹¹ of the mid to late 18th century compared with the preceding and following centuries which had colder temperatures¹¹ and more numerous glacial advances following volcanic eruption. It may also explain the lower number of ice advances during the warmer temperatures of the 12th and 13th centuries² when there was a volcanic activity peak¹, compared with the large number of ice advances following volcanic eruption during the colder temperatures² of the 17th and 19th centuries.

There is evidence that, since the Pleistocene, periods of intense volcanic activity may have coincided with major ice advance phases. There were three well dated major volcanic activity periods¹ in the Southern Andes: from 50–450 BP, from 2150 to 2450 BP and from 4720 to 5450 BP. The dates for the three main Neoglacial ice advance phases have been

listed⁹ as 50–450 BP, 2600–2800 BP and 4500–5000 BP. Two of these Neoglacial phases almost exactly correspond with the Andean volcanic activity periods and the second Neoglacial phase, which peaked from 2600–2800 BP extended from 2300 to 2900 BP (refs 9, 12–17). A fourth Andean volcanic period has been tentatively dated around 8950 BP and there was an ice advance phase^{17–19} around 9200 to 9300 BP.

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Quantitative Spectrochemical Analyses of Feldspars by Ion Bombardment

WHEN the surface of a solid target is bombarded with energetic ions or neutral atoms, spontaneous emission of ultraviolet and visible radiation is observed. When the radiation is analysed by a spectrometer, it is shown to contain characteristic lines of the constituent elements in the solid^{1–4}. It has also been shown that this radiation is caused by the decay of excited atoms ejected from the target surface^{4,5}. The photon emission process is more efficient in insulating targets than metallic targets. This is because of the existence of radiationless de-excitation processes which compete with radioactive decay in the case of metals but not in the case of insulators^{1,6}. The intensities of the spectral lines are directly proportional to the bombarding ion beam current⁷, that is, directly proportional to the number of sputtered particles since the sputtering yield (sputtered atoms per incident ion) for a particular material is invariant if the ion energy is constant providing that the surface conditions do not alter during sputtering. In a previous report¹, one of us suggested that the photon emission phenomenon may provide a new method for chemical analysis of solids, and subsequently White *et al.*³ demonstrated the detection sensitivity, for example 1 part per 10⁶ for sodium in a silicate glass, which could be achieved with this technique. This communication describes some quantitative analyses of a number of feldspars. The analyses were made by comparing the intensities of the emission lines of the major constituents in these materials. In our analyses, we assume that diffusion does not occur to an appreciable extent so that the relative sputtering rates are directly proportional to the atomic concentrations in the target.

Table 1 Feldspar Analyses. Number of Ions on the Basis of Thirty-two Oxygens

	112 (standard)		190			191		297	
	Microprobe	IBSCA	Microprobe	Chemical	Chemical	IBSCA	Microprobe	IBSCA	Microprobe
Si	10.086	9.34	9.423	9.341	9.293	11.23	11.132	9.92	9.990
Al	5.916	6.63	6.523	6.628	6.659	4.75	4.863	6.05	5.995
Fe	0.029	0.037	0.056	0.033	0.059	0.015	0.0	0.026	0.027
Ca	2.005	2.63	2.605	2.628	2.616	1.01	0.903	2.09	2.064
Na	1.720	1.32	1.324	1.248	1.192	2.90	2.939	1.83	1.772
K	0.105	0.050	0.026	0.053	0.028	0.091	0.133	0.074	0.088
An	52.4	65.8	65.8	67.2	68.9	25.2	22.7	52.3	52.6
Ab	44.9	33.0	33.5	31.5	30.3	72.5	74.0	45.8	45.2
Or	2.7	1.2	0.7	1.3	0.7	2.3	3.3	1.9	2.2

	287		109		184		111	
	IBSCA	Microprobe	IBSCA	Microprobe	IBSCA	Microprobe	IBSCA	Microprobe
Si	9.14	9.004	11.79	11.717	11.62	11.805	8.50	8.436
Al	6.81	6.984	4.20	4.297	4.37	4.229	7.46	7.520
Fe	0.038	0.063	0.006	0.0	0.005	0.013	0.038	0.057
Ca	3.04	2.967	0.34	0.298	0.233	0.192	3.52	3.666
Na	0.88	0.937	3.61	3.626	3.14	2.694	0.439	0.250
K	0.076	0.035	0.041	0.011	0.626	0.951	0.041	0.0
An	76.0	75.3	8.5	7.6	5.8	5.0	88.0	93.6
Ab	22.0	23.8	90.3	92.1	78.5	70.2	11.0	6.4
Or	1.9	0.9	1.0	0.3	15.7	24.8	1.0	0.0

Provided that self-absorption is negligible (because the 'vapour' density of absorbing atoms is low), then we have the condition for spectrochemical analyses (intensity is directly proportional to concentration).

The apparatus, which we shall refer to as IBSCA (ion beam spectrochemical analyser⁸), is identical to the one described previously¹ with the exception that in place of the medium quartz spectrograph, the light was analysed in the present work by a grating monochromator with a cooled photomultiplier as detector. The light intensity, being very low, was measured by a standard a.c. chopping technique.

The analysis procedure was carried out as follows. First, the results of the electron microprobe analysis of a particular feldspar 112 was chosen as standard for all the subsequent analyses. Then the ratios of the intensities of Si 2882 : Al 3093, Si 2882 : Al 3944, Si 2882 : Al 3962, Si 2882 : Fe 3737, Ca 3934 : Na 5890, Ca 3968 : Na 5890, Ca 4227 : Na 5890, and K 7665 : Na 5890, were taken. By comparing the intensity ratios of each unknown feldspar with those of 112, we could calculate the concentration ratios. Using the fact that in a unit cell of feldspar there are thirty-two oxygen atoms, sixteen atoms shared by Si, Al and Fe, and four atoms shared by Ca, Na and K, we could work out each individual atomic concentration in terms of thirty-two oxygens. Unfortunately, we could not analyse the oxygen content in the feldspars accurately because of the presence of oxygen impurity both in the argon gas used for producing the ion beam and in the target chamber, possibly due to contamination of water vapour; otherwise the oxygen 7772 line could have been used as a reference line. (This contamination can probably be removed by using a 4A zeolite and more efficient trapping with liquid nitrogen.) We have therefore chosen to carry out our analyses in the manner described rather than comparing intensities of a particular line directly across samples since the present method minimises uncertainties which may arise due to a change in sputtering conditions, for example variations in ion current and chamber pressure. Since the specimen holder in the present apparatus only allows one sample to be analysed at a time, the changing of samples might therefore cause sputtering conditions to vary from one sample to another. The method of analysis adopted, however, assumes only that the sputtering conditions did not vary during an experimental run on a particular sample.

The analyses of seven feldspars are given in Table 1. The electron microprobe analyses by N. Ware (private com-

munication) are also shown for comparison. Electron microscopy has shown that the substructure of these feldspars is on a scale considerably less than 1 μm ^{9,10}. The electron microprobe has a spot size of about 10–100 μm diameter whereas, in IBSCA, the area under investigation was determined by the monochromator slit width which was 150 μm . It is therefore expected that the microprobe and IBSCA should produce similar results. As can be seen from Table 1, the two sets of results are in good agreement. Slight variations in compositions are expected, because different samples (of the same materials) were used for the IBSCA and microprobe analyses. Two chemical analyses¹¹ of feldspar 190 are also shown in Table 1 to demonstrate the degree of uncertainty involved in the determination of the composition of feldspars.

During our investigation, the following conclusions on IBSCA as an analytical instrument have emerged. (1) The apparatus costs considerably less than other comparable analytical instruments because of its simplicity. The estimated cost of constructing such an apparatus is approximately from \$4,000 to \$10,000 depending on the degree of sophistication required. (2) Multi-element analysis is easy to carry out and no elaborate preparation of the sample is required. (3) In the present method, the area of investigation is effectively selected by the variable slit of the monochromator. If the slit can be replaced by a small circular aperture, it will become a microprobe with mechanical scanning facilities. Its ultimate performance as a microprobe is limited by a spatial resolution of $\sim 10 \mu\text{m}$ assuming the photon-emitting sputtered particles leave the target surface with energies $\leq 10 \text{ eV}$. (4) The apparatus can detect light elements down to hydrogen. (5) As the method essentially carries out analysis of the surface, it can be used to measure the concentration profile in a solid as successive surface layers are sputtered away. (6) The line spectra contain very few lines compared to ordinary spectrochemical analysis by arc discharge but more than just resonance lines. This makes identification of elements a very rapid and easy process. (7) With suitable refinements, IBSCA can be used as a research instrument due to its probe characteristics. But as it stands, it can be a very useful field or industrial instrument due to its great simplicity.

In view of the possibility of significant applications, a new IBSCA apparatus using a duoplasmatron ion source to deliver a stable and high current density ion beam and a multiple-target specimen chamber to facilitate rapid and direct com-

parison of intensities is under construction for improved performance.

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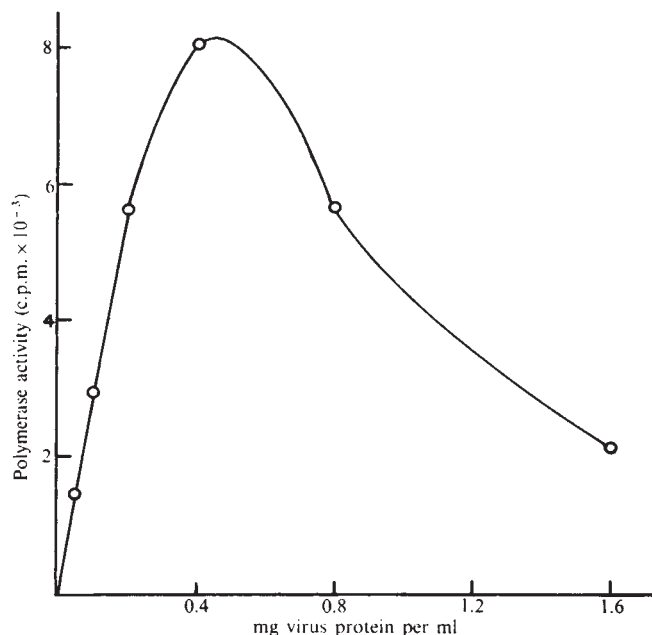


Fig. 1 Virion polymerase activity as a function of virus concentration. The cells, virus sources, and growth of the virus were as described in detail elsewhere⁸. Monolayers of BHK₂₁ cells were infected at a multiplicity of infection of 10 with the Indiana VSV serotype. The media were collected after 12-16 h at 37°C and clarified by spinning at 5,000g for 20 min. All operations were carried out at 4°C. The supernatants were spun at 19,000 r.p.m. for 3 h in a Spinco type 19 rotor. The virus pellets were resuspended in small volumes of 0.05 M Tris-Cl, pH 7.5, buffer and sonicated briefly to disrupt clumps. The sonicates were layered on 12 ml 15-45% sucrose gradients in TK buffer (10 mM Tris-Cl, pH 7.7, 100 mM KCl) and spun at 32,000 r.p.m. for 35 min in the Spinco SW 41 rotor. The single visible band of standard particles (no defective interfering particle bands under these conditions) was collected with a Pasteur pipette and stored on ice until assayed. The concentration of virus protein was determined using the relationship 1 mg virus protein per ml equals 3 A₂₆₀ units (M. E. Reichmann, personal communication). Transcriptase assays were carried out by mixing equal volumes of virus suspension diluted in 25% sucrose in TK buffer with equal volumes of polymerase cocktail. The final concentration of the components in the total reaction mixture was as follows: 50 mM Tris-Cl, pH 7.7; 100 mM KCl; 5 mM MgCl₂; 0.1% mercaptoethanol; 0.02% Nonidet P-40; 1 mM each of ATP, GTP, and CTP; 0.1 mM cold UTP; 2.5 μCi-ml⁻¹ ³H-UTP; and approximately 10-15% sucrose. ³H-UTP incorporation was measured on 50 μl samples after 2 h of incubation at 28°C by the method of Blatti *et al.*⁹ Under these conditions incorporation of 1 nmol of UMP is equivalent to 14,000 c.p.m.

BIOLOGICAL SCIENCE

Inhibitor of vesicular stomatitis virus transcriptase in purified virions

VESICULAR stomatitis virus (VSV) particles contain an RNA-dependent RNA polymerase which gives rise *in vitro* to RNA molecules of the same size and polarity as the mRNA found *in vivo*¹⁻³. It is not clear which structural protein(s) represents the enzymatic activity. Only five proteins (L, G, N, MS, and M, with possibly some additional minor constituents) are detected in the virions⁴, and several studies have suggested that neither the G protein (glycoprotein surface spike) nor the M protein (membrane protein), nor even associated membrane lipids are necessary for transcriptase activity⁵⁻⁷.

Active polymerase has been reconstituted from two inactive fractions, one containing the ribonucleoprotein core and the other, the remainder of the detergent-solubilized proteins⁵. In such a case some components of the virion, in addition to the polymerase itself, may modify or control the transcriptase activity. We report here that VSV particles contain a specific inhibitor of the virion polymerase.

The presence of such an inhibitor was suggested by the results presented in Fig. 1. Polymerase activity measured as a function of virus concentration, was strongly inhibited when virus concentrations were high. This was not due to an early shut-off of *in vitro* activity since under these conditions the kinetics of incorporation were similar for both high and low concentrations, slowing down between 3 and 5 h after the start of the reaction. Various modifications of the reaction cocktail established that none of the components were limiting in inhibiting conditions. Both VSV serotypes—Indiana and New Jersey displayed similar concentration-dependent inhibition, which was the same for virus grown in HeLa as well as BHK₂₁ cells. Further purification of the virus on tartrate-density gradients caused some loss of inhibitory activity for New Jersey VSV but not

for Indiana (see below). The phenomenon could also be demonstrated by adding ultraviolet-irradiated virus (inactive in the polymerase reaction) to standard active virus (Table 1) or by adding defective interfering particles which have little or no transcriptase activity¹⁰ (data not shown). Thus, transcription by the inhibiting virus preparation was not a prerequisite for inhibition.

Preliminary experiments with virus purified through sucrose gradients only, suggested that the inhibitor was serotype-specific. Table 1 shows the results of one such experiment in which Indiana and New Jersey ultraviolet-irradiated virus preferentially inhibited their own serotype polymerase. Several experiments, however, showed either partial specificity or none. Some of this variability may have been caused by contaminating nucleases, which are often present in virus preparations purified on sucrose gradients but not in those density-banded on tartrate gradients (L. P. Villareal, personal communication).

When the virus was purified further through tartrate density gradients the results shown in Fig. 2 were obtained

TABLE 1 Homotypic compared with heterotypic inhibition of *in vitro* transcriptase with sucrose-purified VSV

	c.p.m.
Indiana control	5975
Indiana control + ultraviolet-Indiana	1396
Indiana control + ultraviolet-New Jersey	5390
New Jersey control	5442
New Jersey control + ultraviolet-Indiana	5778
New Jersey control + ultraviolet-New Jersey	2433

Indiana and New Jersey VSV were purified as described in Fig. 1. Virus suspensions in TK-buffered sucrose were irradiated in small nitrocellulose cups at a distance of approximately 6 cm from an ultraviolet sterilizing lamp (G8T5-General Electric) for 6 min. Irradiated virus never incorporated more than 1-2% of controls. Transcriptase assays were carried out as in Fig. 1 except for a final ^3H -UTP concentration of $25 \mu\text{Ci ml}^{-1}$. Control viruses were adjusted to $45 \mu\text{g}$ virus protein per ml and ultraviolet-viruses, to $675 \mu\text{g}$ virus protein per ml final concentration. The c.p.m. listed refer to total incorporation per $30 \mu\text{l}$ sample after 4.5 h at 28°C .

repeatedly. Clearly under these conditions, Indiana VSV polymerase was inhibited specifically by homotypic ultraviolet-irradiated virus but not by heterotypic virus, which was somewhat stimulatory (Fig. 2A). New Jersey VSV polymerase, however, showed a different pattern of response when either serotype was added. Both New Jersey and Indiana ultraviolet-irradiated virus caused early inhibition of incorporation followed later by stimulation (Fig. 2B). Thus, tartrate-purified New Jersey virus did not cause sustained inhibition of either serotype polymerase. It is not known why homotypic inhibition is lost for the New Jersey serotype virus on further purification.

The extent of the inhibition and stimulation of New Jersey polymerase in Fig. 2B, as well as the heterotypic stimulation of Indiana polymerase (Fig. 2A), varied in different experiments, but the homotypic Indiana inhibition was always considerable and readily observed. It is not clear whether these various phenomena are reflections of one or more activities in the preparations.

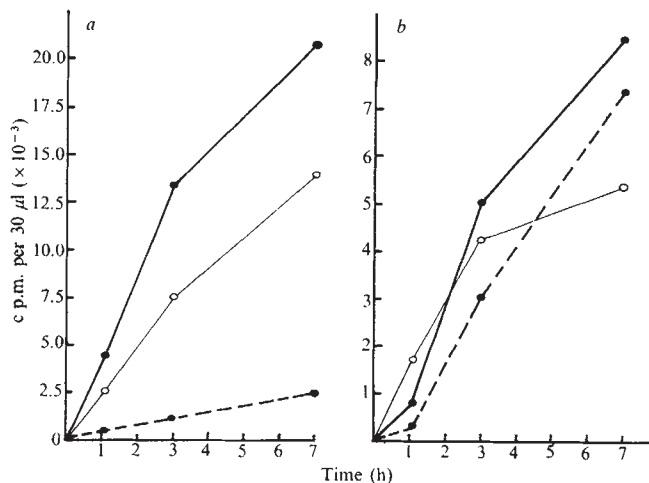


FIG. 2 Serotype specificity of transcriptase inhibition with tartrate-purified virus. Indiana and New Jersey VSV were further purified by layering on 15-45% NaK-tartrate gradients and spun in the Spinco SW 41 rotor at 37,000 r.p.m. for 3 h at 4°C . The virus bands were recovered and dialysed against TK buffer. Transcriptase assays were carried out as in Table 1. Control preparations contained $50 \mu\text{g}$ virus protein per ml and the ultraviolet preparations were added to a final concentration of $800 \mu\text{g ml}^{-1}$. A, Indiana polymerase; B, New Jersey polymerase; $\circ$, control; $\bullet$ —, plus ultraviolet-irradiated New Jersey virus; — $\bullet$ —, plus ultraviolet-irradiated Indiana virus.

The effect of graded amounts of ultraviolet-irradiated virus was examined in experiments carried out in parallel to those of Fig. 2. The totals after 7 h of incorporation were used to construct the curves shown in Fig. 3. In agreement with the previous results, Indiana showed strong homotypic inhibition, while New Jersey polymerase was not inhibited by either serotype. The inhibition reached a maximum of approximately 80% at a particle ratio of approximately 5 ultraviolet-irradiated virus:1 active virus. On the other hand, stimulation of polymerase activity did not reach a maximum with increasing amounts of irradiated virus.

We conclude that the inhibitory activity has some type specificity. It is therefore likely that this phenomenon is

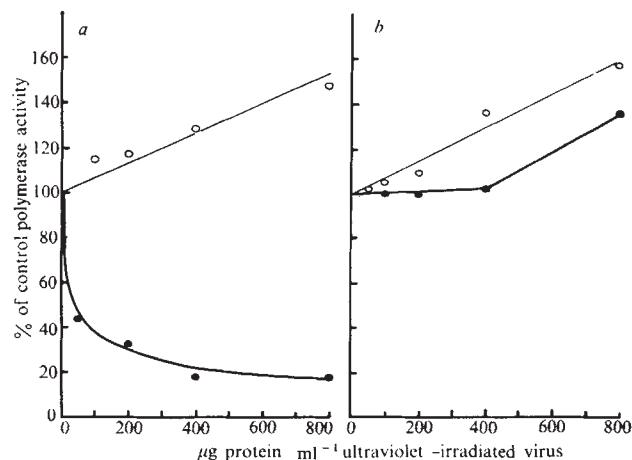


FIG. 3 Polymerase activity versus ultraviolet-virus concentration. The activities plotted are explained in the text and in the legend for Fig. 2. $\circ$, plus ultraviolet-irradiated New Jersey virus; $\bullet$, plus ultraviolet-irradiated Indiana virus.

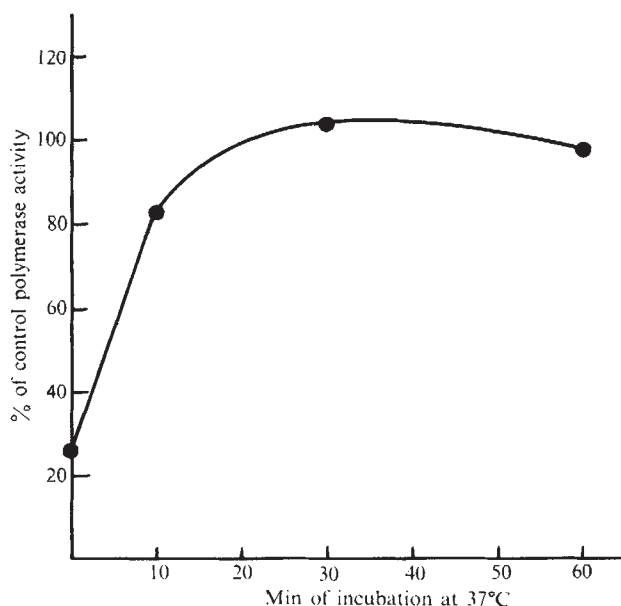


FIG. 4 Loss of inhibitor activity at 37°C in the presence of Nonidet P-40 detergent. Indiana VSV purified through sucrose and tartrate gradients was ultraviolet-irradiated as before and incubated at 37°C in TK buffer with 0.02% Nonidet P-40 at a concentration of $800 \mu\text{g}$ virus protein per ml. This suspension was then assayed for inhibitor activity as described in Fig. 2 at a final concentration of $400 \mu\text{g}$ per ml. The totals after 6.25 h of incorporation were used to construct the curve shown in the figure.

TABLE 2 Effects of predigesting virions with trypsin on polymerase and inhibitor activities of Indiana VSV

	c.p.m.
Control virus 60 $\mu\text{g ml}^{-1}$	4,309
+ 65 $\mu\text{g ml}^{-1}$ ultraviolet-virus	3,387
+ 130 $\mu\text{g ml}^{-1}$ ultraviolet-virus	2,630
+ 260 $\mu\text{g ml}^{-1}$ ultraviolet-virus	1,463
+ 65 $\mu\text{g ml}^{-1}$ trypsinized ultraviolet-virus	3,009
+ 130 $\mu\text{g ml}^{-1}$ trypsinized ultraviolet-virus	2,303
+ 260 $\mu\text{g ml}^{-1}$ trypsinized ultraviolet-virus	1,629
Trypsinized virus 60 $\mu\text{g ml}^{-1}$	3,367

A 0.2 ml suspension of sucrose-purified Indiana VSV containing 2.5 mg virus protein per ml and 100 $\mu\text{g ml}^{-1}$ trypsin was incubated at 37°C for 2 h. The suspension was then passed through a 10 ml column of Bio-Gel A-5m, 100–200 mesh (Bio Rad), equilibrated with TK buffer. Control virus was handled in the same way except that trypsin was added after the 2 h incubation at 37°C. The transcriptase assays were carried out as in Table 1. The c.p.m. listed refer to incorporation per 30 μl samples after 3 h at 28°C. The kinetics of incorporation were similar to those shown in Fig. 2A for both control and trypsinized virus.

controlled, directly or indirectly, by some genetically determined virus component.

Initial investigations of the heat stability of the Indiana inhibitor gave the following results. Purified virions suspended in TK-buffered sucrose solution showed no loss of inhibitory activity when incubated for 2 h at 37°C. A temperature of 100°C for 1 min under the same conditions destroyed the inhibitor. If the virus was first disrupted with 0.02% Nonidet-P40 and incubated at 37°C as above, the inhibitor activity was lost with a half-life of approximately 5 min (Fig. 4), suggesting that the inhibitor irreversibly modifies a component of the polymerase under the conditions of the *in vitro* assay.

To determine whether the inhibitory activity is present on the surface of the virion, possibly as the spike protein,

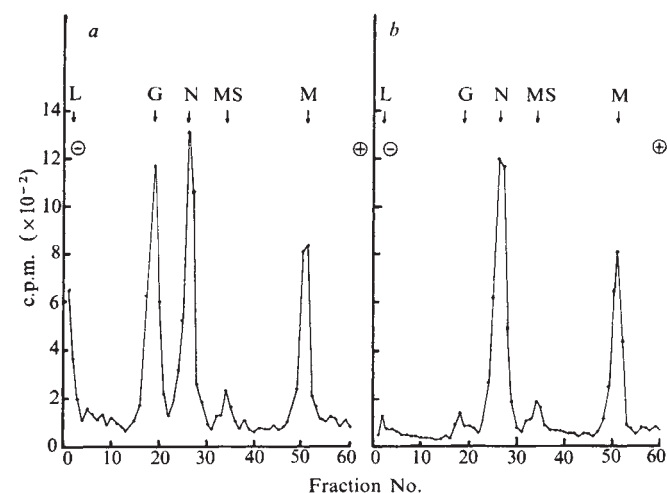


FIG. 5 SDS gel electrophoresis of control and trypsinized Indiana VSV. Identical samples to those used in Table 2 were mixed with a small amount of high specific activity ^3H -amino acid (phenylalanine, tyrosine, and valine)-labelled Indiana VSV (supplied by Dr John Mudd). After processing as in Table 2, the samples were mixed with SDS and mercaptoethanol to 1% final concentration. They were then boiled for 30–60 s and dialysed against 10 mM sodium phosphate buffer, pH 6.8, 0.1% mercaptoethanol, 0.1% SDS, and boiled again. 0.3 ml samples were then subjected to electrophoresis in 7.5% acrylamide, 0.37% bis-acrylamide gels, in 0.1 M sodium phosphate, pH 6.8, 0.1% SDS buffer, for approximately 15 h at 90 V. The gels were crushed in a linear fractionator and the fractions counted in a Triton X-100 scintillation cocktail. A, control; B, trypsin-treated.

the Indiana virus preparation was digested with trypsin to remove the surface glycoprotein without affecting other virus components¹¹. The resultant skeleton was separated from trypsin by passing through a small column of Bio-Gel A-5m. This structure was then tested for remaining polymerase and inhibitory activity. Table 2 shows that approximately 80% of the polymerase activity and essentially all the inhibitory activity was retained in the skeleton structure. Parallel controls, in which a small amount of high specific activity ^3H -amino acid-labelled virus was added before trypsin digestion, were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Fig. 5 indicates that more than 90% of the glycoprotein was removed. This experiment provides further evidence that the inhibitor is a virus-specific component, for trypsin treatment probably removes surface contaminants. Furthermore, the intact glycoprotein is ruled out as a candidate for the cause of the inhibitory activity.

We conclude from these data that purified VSV contains a specific inhibitor of the virus transcriptase. Two lines of evidence support this conclusion. First, the inhibition shows some degree of serotype specificity, and second, the inhibitor is present within the core structure of the virion produced by trypsin digestion. It is clear that in many studies where VSV transcriptase was measured *in vitro*, this inhibitory activity must have been present.

We can only speculate about the role of this activity *in vivo*. If the inhibitor is a VSV protein, as is most likely, the synthesis of this protein in the infected cell could lead to a switch from transcriptase to replicase activity. The common polymerase 'core' hypothesis for these two distinct activities is attractive as no new major proteins have been detected in infected cells apart from structural proteins^{12,13}. Another role for this activity could be to shut off host cell RNA synthesis.

It is interesting that Astell *et al.*¹⁴ reported that a reo-virus structural protein, σ_3 , inhibits reovirus transcriptase when added back to subviral preparations lacking this protein. The relationship, if any, between these different inhibitors is not clear.

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Structural homology of the glutamine amidotransferase subunits of the anthranilate synthetases of *Escherichia coli*, *Salmonella typhimurium* and *Serratia marcescens*

ANTHRANILATE synthetase (AS), the enzyme catalysing the initial reaction unique to tryptophan biosynthesis, chorismate + glutamine $\rightarrow$ anthranilate, has been characterised from a number of bacterial genera¹. In *E. coli*²⁻⁴, *Salmonella typhimurium*⁵⁻¹⁰, and *Aerobacter aerogenes*¹¹, this enzyme and anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase (PRT) exist in a complex that also performs the second reaction of tryptophan biosynthesis, the conversion of anthranilate + 5-phosphoribosyl-1-pyrophosphate $\rightarrow$ phosphoribosyl anthranilate. The AS-PRT complex of these bacterial genera is composed of two non-identical polypeptide components, each with a molecular weight of approximately 60-65,000. Component I of the complex can convert chorismate + NH₃ $\rightarrow$ anthranilate but cannot use glutamine as amino donor. Component II of the complex is bifunctional; it provides the glutamine amidotransferase (GAT) and the PRT activities. In *Serratia marcescens*¹²⁻¹⁴, *Bacillus subtilis*¹⁵⁻¹⁶, *Acinetobacter calcoaceticus*¹⁷⁻¹⁸, and various species of *Pseudomonas*¹⁹⁻²⁰, AS is not associated with PRT, which is a distinct protein. The AS of these bacteria consists of two non-identical subunits of different sizes. The large subunit, approximately 60-70,000 in molecular weight, functions as does component I of *E. coli*, *S. typhimurium* and *A. aerogenes* while the small subunit contributes the GAT activity. The molecular weights of the GAT subunit of *S. marcescens*^{12,14}, *Pseudomonas putida*^{19,20}, *B. subtilis*¹⁵, and *A. calcoaceticus*¹⁸ have been estimated to be 21,000, 18,000, 16,000 and 14,000 respectively. The PRT of *S. marcescens* has a molecular weight of 45,000, which is smaller than that of component II of *E. coli* and *S. typhimurium* by an amount equivalent to the molecular weight of a GAT subunit¹⁴.

The two functions of the component II of *E. coli* and *S. typhimurium* have been shown to be associated with different segments of the polypeptide chain. Proteolytic digestion of either the component I-component II complex

of *E. coli* and *S. typhimurium*⁹ or purified component II of *S. typhimurium*²¹ destroys PRT activity, but produces a component II fragment with GAT activity which is similar in size to the GAT subunit of *S. marcescens*. Genetic and mutational studies have further indicated that the amino terminal third of the component II of *E. coli*²² and *S. typhimurium*²¹ is responsible for the GAT activity while the remaining two thirds of the polypeptide provides PRT activity^{21,23}.

It has been suggested^{12,14,21} that the bifunctional component II of *E. coli*, *S. typhimurium* and *A. aerogenes* arose by the fusion of two genes, one coding for a GAT and the second coding for PRT. To examine this possibility, we have isolated and compared the amino terminal sequences of the GAT subunit of *S. marcescens* and the component II of *E. coli* and *S. typhimurium*.

For ease in purification and subsequent subunit separation, we isolated the GAT portion of component II of *E. coli* and *S. typhimurium* from partial proteolytic digests of intact component I-component II complexes. As indicated below, the amino terminal sequence of the GAT portion of the component II of *E. coli* is identical with that of intact component II of this organism. Presumably this is also true of *S. typhimurium*. The AS of *S. marcescens* was isolated intact and the subunits then separated. The details of the purification of the component II fragments and the *S. marcescens* complex and subunits will be described elsewhere. On the basis of SDS gel electrophoresis analyses we estimate that the molecular weights of the component II fragments of *E. coli* and *S. typhimurium* are 23,000 while that of the GAT subunit of *S. marcescens* is 21,400. The molecular weight of the component II fragment of *S. typhimurium*^{9,21} had previously been estimated as 24,000 and that of the GAT subunit of *S. marcescens*¹² as 21,000.

Automatic Edman degradation of the purified polypeptides was performed with a Beckman sequencer²⁴. Phenylthiohydantoin-amino acids were identified by gas-liquid chromatography²⁵ and (or) amino acid analysis²⁶ after hydrolysis of phenylthiohydantoin-derivatives with HCl or HI. The amino acid sequences of the component II fragments of *E. coli* and *S. typhimurium* and the intact GAT subunit of *S. marcescens* deduced from several degradation runs are shown in Fig. 1.

	5	10	15	20
<i>E. coli</i>	Ala- Asp- Ile - Leu- Leu- Leu - Asp- Asn- Ile - Asp- Ser- Phe- Thr- Tyr- Asn- Leu- Ala- Asp- Gln- Leu-			
<i>S. typhimurium</i>	- - - - - - - - - - - - - - Trp - - - - - - -			
<i>S. marcescens</i>	- - - - - - - - Val - - - - - - - - Val - - - -			
	25	30	35	40
	Arg- Ser- Asn- Gly- His- Asn- Val- Val- Ile - Tyr- Arg- Asn- His- Ile- Pro- Ala- Gln- Thr- Leu- Ile -			
	- Thr -			
	- Ala Ser - - - Gln - - - - - - - - Gln - Gly - - - Val Ile -			
	45	50	55	60
	Glu - Arg- Leu- Ala- Thr- Met- Ser- Asn- Pro- Val- Leu- Met- Leu- Ser- Pro- Gly- Pro- Gly- Val- Pro- Ala-			
	Asp - - - - - ? - - - - - X X X X X X X X X			
	- - - Gln Gln - ? Gln - - - - - - - - - - - Ala - Val			

FIG. 1 Comparison of the amino-terminal sequences of the component II fragments of *E. coli* and *S. typhimurium* and AS-GAT subunit of *S. marcescens*. Residues of the *S. typhimurium* or *S. marcescens* proteins which differ from those in *E. coli* are given, while identity to the *E. coli* residue is indicated by a dash (-). Automatic Edman degradation of the component II fragment of *S. typhimurium* was programmed for 52 cycles only, while 61 residues were sequenced with the proteins of *E. coli* and *S. marcescens*. The amino acid residue at position 47 in the *S. typhimurium* and *S. marcescens* sequences could not be identified.

(Details will be published elsewhere.) To establish that the sequences of the component II fragments are identical to the amino terminal sequences of intact component II, we performed fifteen steps of automatic Edman degradation on isolated, intact, component I-component II complex of *E. coli*. The sequence attributable to component II was identical to that of the component II fragment.

It is evident from the alignment in Fig. 1 that the three sequences are homologous. The sequences of *E. coli* and *S. typhimurium* differ from that of *S. marcescens* by 23% (14/60 residues) and 27% (14/51 residues), respectively. It can also be seen that the sequences are in the same register throughout the 51 residue region compared, and that all three begin with the identical sequence of 8 residues. In view of these similarities it seems likely that the genes for the three GAT regions have homologous nucleotide sequences in the regions coding for the amino terminal segment of the respective polypeptides. Thus, it is highly probably that the GAT region of the bifunctional component II polypeptide of *E. coli* and *S. typhimurium* had the same evolutionary origin as the GAT subunit of the AS of *S. marcescens*. Functional homology of the GAT subunits of *E. coli* and *S. marcescens* is demonstrated by the observation that the GAT subunit of the latter species will complement *E. coli* component I to form an active hybrid molecule that can use glutamine as an amino donor¹⁴. In some organisms, for example *B. subtilis* and *A. calcoaceticus*, in which AS contains a small GAT subunit and is not associated with PRT, the GAT subunit appears to participate in a second biosynthetic reaction, that of *p*-aminobenzoate formation^{16,18}. It is not known if this is also true of the GAT subunit of the AS of *S. marcescens*. In view of all these observations, we can hypothesize, in agreement with earlier suggestions^{12,14,21}, that the evolutionary path resulting in the development of the bifunctional component II of *E. coli* and *S. typhimurium* was as follows. In an ancestral organism tryptophan biosynthesis preceded with NH₂ rather than glutamine as amino donor in anthranilate formation. Subsequently a GAT subunit, perhaps involved in *p*-aminobenzoate synthesis, acquired the ability to complex with a polypeptide analogous to AS component I, thereby permitting the use of glutamine, a more suitable amino donor. Then a copy of the gene for this GAT subunit was translocated into a tryptophan operon, adjacent to the gene coding for PRT. Coincident with the integration event, or later, the GAT and PRT genes became fused.

Two additional observations may be cited which lend support to some of the stages in the above scheme. First, in *E. coli*²⁷ it seems that there is a distinct gene for the GAT subunit of the *p*-aminobenzoate synthetase complex. Second, the GAT region of the gene coding for component II of *E. coli* is not essential for anthranilate formation^{23,28,29}. This suggests that addition of glutamine-utilizing activity to AS simply increased the efficiency of conversion of chorismate to anthranilate rather than providing the ability to perform a new, essential biosynthetic reaction.

It is not yet known whether the structural gene for the GAT subunit of the AS of *S. marcescens* is in the same operon as the structural genes for either the large subunit of AS, or for PRT¹⁴. In *B. subtilis* the structural gene for the small GAT subunit is unlinked to a single operon containing the genes for all the other proteins involved in tryptophan biosynthesis¹⁶. In *A. calcoaceticus* the structural gene for the GAT subunit is in a gene cluster also containing the genes for PRT and indole glycerolphosphate synthetase¹⁷. This gene cluster is not linked to the structural genes for any of the other proteins of tryptophan biosynthesis¹⁷. In *E. coli* and *S. typhimurium* all the genes are in one operon and the gene for component II follows immediately after the gene for component I, with the genetic region specifying the GAT portion of component

II, the amino terminal third of this polypeptide, closest to the gene for Component I^{5,21,22}.

It should be noted that the fusion of two structural genes with the retention of both enzyme activities has been demonstrated in studies with the histidine operon of *S. typhimurium*³⁰. Both fused and separate forms of functional polypeptides occur for other reactions of tryptophan biosynthesis e.g., in *E. coli*³¹⁻³³, *S. typhimurium*³³, *A. aerogenes*³³ and *S. marcescens*^{34,35}, phosphoribosylanthranilate isomerase and indoleglycerol phosphate synthetase activities are associated with a single polypeptide chain while in *A. calcoaceticus*¹⁷, *B. subtilis*³⁶ and *Pseudomonas putida* these activities reside in separate polypeptide chains.

A final point seems pertinent. The postulated series of evolutionary stages—from no GAT, to one GAT participating in two pathways, to two GATs, each specific for a pathway, can be considered analogous to the evolution in higher organisms of multiple genes coding for functionally and structurally similar proteins.

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DNA Repair Monitored by an Enzymatic Assay in Multinucleate Xeroderma Pigmentosum Cells after Fusion

XERODERMA pigmentosum is a rare cutaneous disease of man displaying an autosomal recessive pattern of inheritance¹. The most conspicuous clinical symptom exhibited by homozygously afflicted individuals is hypersensitivity of the skin to solar radiation. Typically, the disorder eventuates in a high incidence of multiple carcinomas and frequently becomes terminal after the onset of metastatic epithelioma². Two clinical syndromes characterise the disease: (i) the more common classical form manifested solely by the cutaneous abnormalities and (ii) the De Sanctis-Cacchione (DSC) syndrome in which severe neurological complications, including mental retardation, accompany the skin malignancies².

Ultraviolet radiation ($\lambda < 320$ nm) induces cyclobutyl dimers between adjacent pyrimidines in the DNA of human cells. Cumulative evidence³ indicates that these otherwise deleterious photoproducts are removed from cellular DNA by an excision-repair process. The repair mechanism probably proceeds by the following sequence of enzymatic steps: (i) intrastrand incision proximal to the photoproduct; (ii) excision of the lesion perhaps within an oligonucleotide; (iii) repair replication to fill in the resulting single-strand gap, and (iv) strand rejoining³. Excluding several unexplained cases^{4,5}, primary fibroblasts derived from many genetically unrelated patients with either form of xeroderma pigmentosum are deficient in the excision repair of ultraviolet-induced damage^{3,6-9}. By analogy to the well documented excision-repair properties of ultraviolet-sensitive bacteria, the primary molecular defect in xeroderma pigmentosum most likely impairs the functioning of an ultraviolet-specific endonuclease¹⁰⁻¹³—the human analogue of the repair enzyme mediating the initial incision reaction in the bacterium *Micrococcus luteus*¹⁴.

In spite of an apparent obstruction at the same stage in repair, the genetic defects in the two clinical forms of xeroderma pigmentosum are complementary. Using somatic cell hybridisation, de Weerd-Kastelein *et al.*¹⁵ demonstrated that in binuclear cells containing one nucleus from each clinical form, DNA repair returns to that found in normal, repair-proficient cells. Thus, the ultraviolet-induced response is normal only when the binuclear cells possess a heterozygous combination of nuclei. To gain further insight into the fidelity of complementation, particularly at the biochemical level, we have used an *in vitro* enzymatic assay¹² to follow the disappearance of dimer-containing sites from the DNA of ultraviolet-damaged, multinucleate heterokaryons of xeroderma pigmentosum strains. The rationale behind the assay is the selective ability of an ultraviolet-specific endonuclease purified from *M. luteus* to produce single-strand scissions at sites containing pyrimidine dimers (referred to as nuclease-susceptible sites) in DNA extracted from ultraviolet-damaged cells.

As strongly suggested by inferential evidence¹², the assay monitors the presumed initial incision event. Use of this assay in combination with one measuring a late step in repair (that is, repair replication) may therefore permit us to detect in xeroderma pigmentosum hybrids any peculiarities in the excision-repair process stemming from the occurrence of genetic complementation.

The experiments reported here were performed with three human strains: AH⁹, established from a healthy volunteer; XP4RO derived from a classical xeroderma pigmentosum patient and XP25RO¹², procured from a De Sanctis-Cacchione donor. Monolayer cultures of primary fibroblasts were cultured at 37° C in Roux flasks containing thymidine-free F12 medium¹⁶ supplemented with 15% (v/v) foetal calf serum (Gibco). When required, radionuclides were incorporated into endogenous DNA by incubating cultures for 36–48 h in growth medium containing either 0.5 $\mu\text{Ci ml}^{-1}$ ³H-methyl thymidine (specific activity 2 Ci mmol⁻¹) or 1.0 $\mu\text{Ci ml}^{-1}$ 2-¹⁴C-thymidine (specific activity 53 mCi mmol⁻¹). (Both radiochemicals were purchased from the Radiochemical Centre, Amersham, Great Britain.)

Multinucleate cells were formed according to the procedure of Harris and Watkins¹⁷. Trypsinised cells of the two chosen strains were mixed in a 1:1 ratio at a final concentration of 2×10^6 cells per ml and incubated with ultraviolet-inactivated Sendai virus (final titre 500 haemagglutinating U ml⁻¹). This mixture was kept for 4 min at 4° C and after shaking at room temperature incubated for 20 min at 37° C. The fused fibroblasts were then seeded in Falcon Petri dishes (diameter 9 cm, each dish receiving the equivalent of 2×10^6 single cells) and incubated overnight at 37° C.

All subsequent procedures including those pertaining to (i) the administration of far ultraviolet light (chiefly 254 nm radiation; incident exposure rate 9 ergs mm⁻² s⁻¹); (ii) the enzymatic assay to monitor the disappearance *in vivo* of dimer-containing sites from cellular DNA, and (iii) ultraviolet-induced repair replication as measured by isopycnic centrifugation in sodium iodide gradients, were performed as described in detail earlier^{12,18}.

Incubation of ultraviolet-irradiated cultures for selected periods before performing the *in vitro* enzymatic assay enables us to trace the time course for the disappearance of nuclease-susceptible sites from the DNA of XP4RO/XP25RO hybrids and thus determine whether genetic complementation occurs at the presumed initial step in excision repair. The results of such an experiment are presented in Fig. 1. Fused cultures containing XP4RO/XP25RO heterokaryons can indeed rid their DNA of nuclease-susceptible sites; for example, after exposure to 125 ergs mm⁻² of germicidal light ~35% of the sites initially produced have been removed in 15 h while a smaller percentage—up to 20%—is eliminated during the same interval following an incident fluence of 250 ergs mm⁻². In sharp contrast, non-complementary preparations of XP4RO/XP4RO and XP25RO/XP25RO, like the corresponding unfused cell cultures¹², are at most only marginally proficient at attacking these sites containing pyrimidine dimers. It would appear, however, that the capacity for eliminating the sites from ultraviolet-damaged DNA has only partially returned after cell fusion of XP4RO was XP25RO; that is by the fifteenth hour after irradiation with 125 erg mm⁻² the xeroderma pigmentosum hybrid population has removed ~50% of those eliminated by normal cells. This intermediary level of site removal observed in the XP4RO/XP25RO hybrids is in excellent agreement with the percentage of the total nuclei in the same fused cultures present in multinucleate cells (that is ~55%). And since the enzymatic assay monitors not just the complementary heterokaryons but rather the entire cell population (also comprising mononucleate unfused cells and non-complementary fused ones containing two or more nuclei derived from the same strain), it seems reasonable to conclude that within the sub-population of XP4RO/XP25RO heterokaryons the ability to execute site removal has been restored to that proficiency displayed by normal cells.

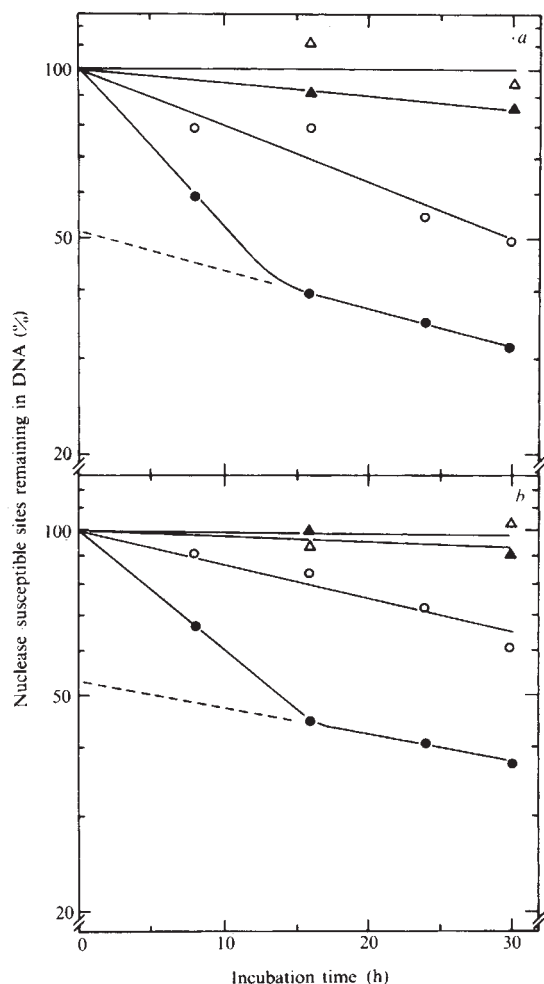


Fig. 1 Relative rate of disappearance of nuclease-susceptible sites from the DNA of various fused human cultures after exposure to measured fluences (*a*, 125 ergs mm⁻²; *b*, 250 ergs mm⁻²) of ultraviolet radiation. Tritium-labelled cells of two selected strains were artificially fused as were two parallel ¹⁴C-labelled preparations. After incubation overnight 254 nm light was administered to the ³H-labelled hybrid cultures; immediately thereafter both the ultraviolet-damaged and the undamaged cultures were incubated in pairs for requisite times. The two types of radioactive cells were collected, pooled, and mixed thoroughly before cell lysis and *in toto* extraction of the irradiated and un-irradiated DNA. Two aliquots of the purified DNA were next incubated under conditions described before¹², one sample in the absence and the other in the presence of the ultraviolet-specific endonuclease from *M. luteus*. The number of nuclease-susceptible sites in the ultraviolet-injured DNA was determined retrospectively by sedimenting the two incubated DNA samples through alkaline sucrose gradients (40,000 r.p.m. at 20° C for 150 min in a SW 50.1 rotor). Finally the ensuing DNA profiles were analysed by a digital PDP8/1 computer to quantify the incidence of single-strand scissions directly attributable to endonucleolytic attack by the *Micrococcus* enzyme. (See ref. 12 for details concerning this computation.) The data for each curve were normalised by expressing the number of sites detected in the incubated samples as a percentage of the value (taken as 100%) found in the corresponding un-incubated sample. Each experimental point is the arithmetic mean of at least two independent determinations. Combinations of fused strains: ●, AH/AH; ○, XP4RO/XP25RO; ▲, XP4RO/XP4RO; △, XP25RO/XP25RO.

Self-hybridisation of a single strain (that is XP4RO to XP4RO) does not noticeably alter the repair properties of human fibroblasts. In unfused cell cultures the repair-proficient AH strain displays two-component kinetics for site removal at fluences of 65 and 125 but not at 250 erg mm⁻² (ref. 12), but in fused cell populations of AH these kinetics are also readily

demonstrable at 250 erg mm⁻² (Fig. 2). Although these discrepancies are of little relevancy to the interpretation of our data, they suggest a difference in the general morphological geometry of the fibroblasts after heterokaryosis. In comparison with the mononucleate cells, the fused cells, particularly those of high multinucleicity, attach but do not spread out on the Petri dish to the same degree before ultraviolet irradiation with the result that the effective biological fluence (measured retrospectively by the enzymatic assay) is consistently less than the calibrated incident fluence. This dosimetrical discrepancy presumably reflects partial shielding of nuclei by various cellular constituents absorbing 254 nm radiation.

In the same experiment we also studied complementation between strains representing the classical xeroderma pigmentosum and the DSC syndromes by assessing in the fused cell populations the magnitude of repair replication. This indicator of repair activity is interpreted to reflect the cumulative occurrence of the third step in excision repair in which single-strand 'repair patches' are inserted at infrequent intervals along the damaged DNA molecule⁶.

Fused preparations of normal AH cells served to simulate hybrids in which the extent of complementation was maximal while hybrid cultures of XP4RO/XP4RO and XP25RO/XP25RO were assayed to establish the residual level of repair exhibited by each XP strain in the absence of complementation. Figure 2 clearly demonstrates that XP4RO and XP25RO cells are considerably more proficient at repair replication when they are combined in multinucleate heterokaryons than when self-fused in homokaryons. As suggested above to account for the partial restoration of site

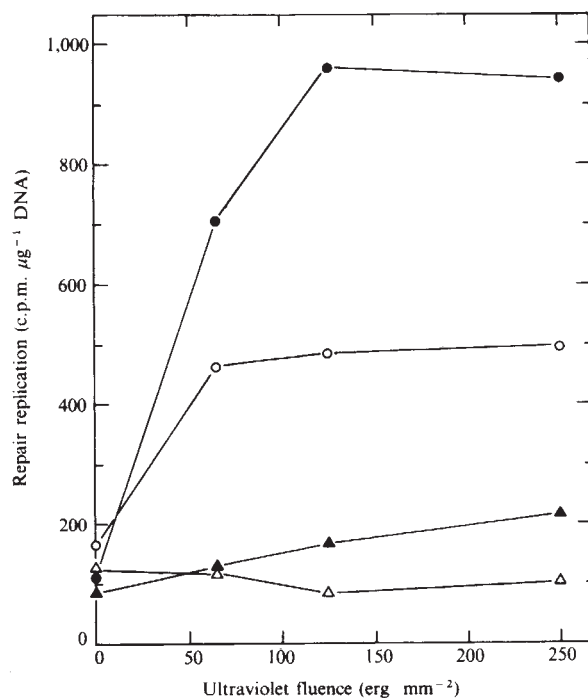


Fig. 2 Relative levels of DNA repair replication in artificially fused human fibroblasts as a function of incident ultraviolet fluence. After cell fusion and incubation overnight, the unlabelled cultures were grown in BrUdR (2 μg ml⁻¹) and FUdR (10⁻⁶ M) for 2 h, exposed to indicated fluences of 254 nm radiation, and immediately labelled with ³H-methyl TdR (10 μCi ml⁻¹), BrUdR (2 μg ml⁻¹), FUdR (10⁻⁶ M) and hydroxyurea (10⁻³ M) for an additional 3 h. The DNA was extracted *in toto* from each sample and centrifuged to equilibrium in neutral NaI gradients (38,000 r.p.m. at 20° C for 44 h in a Type 40 fixed-angle rotor). The isopycnic gradients were generated and analysed to measure the amount of repair replication (expressed as specific activity, that is, c.p.m. μg⁻¹ DNA, in DNA of normal density). Combinations of fused strains: ●, AH/AH; ○, XP4RO/XP25RO; ▲, XP4RO/XP4RO; △, XP25RO/XP25RO.

removal in the same preparations, the fractional amount of repair replication detected in the XP4RO/XP25RO hybrid cultures compared with that found in normal cells is an effect ascribed to the fusion condition in which only a fraction of the nuclei are present in multinucleate heterokaryons. Hence, in accord with similar studies reported elsewhere^{15,19}, after artificial union classical xeroderma pigmentosum and DSC strains mutually compensate for their respective genetic deficiencies.

Our results strongly suggest that as a result of genetic complementation multinucleate hybrid fibroblasts of XP4RO and XP25RO, human strains derived from classical xeroderma pigmentosum and DSC donors, respond to insult from ultraviolet radiation by executing *bona fide* excision repair with complete fidelity. These heterokaryons seem to exhibit normal kinetics for both the disappearance of nuclease-susceptible sites and repair replication, parameters probably measuring initial and late steps in excision repair. Furthermore, there is no rampant accumulation of single-strand scissions in the DNA of the damaged hybrid cultures during post-radiation incubation, as the incidence of strand breaks in ultraviolet-damaged DNA from incubated as compared with non-incubated samples was negligible. The most reasonable interpretation of our data is that the two clinical forms of xeroderma pigmentosum reflect mutations in different genetic complementation groups coding for the initiation of excision repair.

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Measurement of Defects in Ultraviolet-irradiated DNA by the Kinetic Formaldehyde Method

ULTRAVIOLET irradiation of DNA converts a portion of the adjacent pyrimidine bases into cyclobutane dimers of the form $\hat{C}\hat{C}$, $\hat{C}\hat{T}$ and $\hat{T}\hat{T}$ ¹. As a consequence, hydrogen bonding and base stacking at these sites are disrupted, and localised denatured regions may appear. (Ultraviolet-induced denaturation has been reviewed in ref. 2.) Treatment of DNA with formaldehyde results in a preferential reaction at these denatured sites (or defects) because formaldehyde reacts only with free but not hydrogen-bonded amino groups³.

The theory developed by Trifonov *et al.*⁴ and Lazurkin *et al.*⁵ called the kinetic formaldehyde method (KF method), relates the rate of the reaction of formaldehyde with DNA to the concentration of defects in the DNA. Since formaldehyde unwinds the DNA as it reacts, the rate of reaction can be followed by determining the change in helicity from the change in the absorbance A_t at some time t , where

$$\theta = (A_{\text{final}} - A_t) / (A_{\text{final}} - A_{\text{initial}})$$

in which A_{initial} and A_{final} are the initial and final absorbances respectively. The relationship between θ and t is given by

$$(-\ln \theta) / t = 2cv + pvt$$

where c is the concentration of initial defects per base pair, v is the velocity of unwinding at a defect site, and p is the rate constant for the initiation of new defect sites. A plot of $(-\ln \theta) / t$ against t gives a straight line with slope pvt and intercept of $2cv$ on extrapolation to $t=0$. The initial rate of unwinding is defined as the intercept, $I=2cv$.

Defects can be single or double strand chain breaks or pyrimidine dimers. Experimental evidence shows that v is the same for both single and double strand breaks⁵. Little direct evidence, however, is available to support the basic assumption made in those studies that v for pyrimidine dimers is the same as for chain breaks, and the assumptions made in calibrating the KF method for pyrimidine dimers have been criticised².

Shafranovskaya *et al.*⁶ showed that for ultraviolet (254 nm) fluences $>10 \text{ J m}^{-2}$ the rate of formaldehyde reaction with irradiated DNA was six times slower than expected on the basis of numbers of dimers present in the DNA. The result was interpreted in terms of clustering of the dimers such that six dimers, for example, would be expressed as a single defect site. The origin of such clustering was proposed to be long range energy transfer (over as many as several thousand base pairs) resulting in preferential de-excitation at or near the site of an initially formed pyrimidine dimer, which serves to block further migration of the energy. An alternative possibility is that a break and a dimer are not equivalent sites, because of the constraints in unwinding an unbroken helix, and hence react with formaldehyde at very different rates.

We have designed experiments to test the assumption used by Shafranovskaya and coworkers that dimer and chain break defect sites react with formaldehyde at the same rate. To do so we used ultraviolet-endonuclease to convert pyrimidine dimer sites into chain breaks. This enzyme recognises pyrimidine dimers and makes nicks at the dimer sites⁷⁻⁹. A comparison of the rates of formaldehyde reaction before and after enzyme treatment should demonstrate any differences between the velocity of unwinding, v , associated with dimers and with chain breaks.

Tritium-labelled DNA was isolated from *Escherichia coli* B3 by the method of Marmur¹⁰ and dissolved in 0.01 M phosphate buffer, pH 7. Irradiation was done with the output (mostly 254 nm) from a 15-W low-pressure mercury lamp, in some cases in the presence of 0.03% H_2O_2 to produce chain breaks. Photolysis of H_2O_2 occurs with a quantum yield of unity, creating hydroxyl radicals which in turn make single strand

Table 1 Kinetic Formaldehyde Analysis of DNA containing Chain Breaks and Dimers

Ultraviolet fluence (J m ⁻²)	H ₂ O ₂	M_w (daltons $\times 10^{-6}$)	Breaks per base pair ($\times 10^3$)	$\hat{U}T/T$ ($\times 10^2$)	$\hat{T}T/T$ ($\times 10^2$)	PyPy per base pair ($\times 10^3$)	I (min ⁻¹ $\times 10^3$)	$V=I/2C$ (min ⁻¹)
0	+	7.2	0	—	—	—	0	0
100	+	2.5	0.33	—	—	—	1.75	2.7 $\pm$ 0.5
500	+	0.63	1.93	—	—	—	6.35	1.7 $\pm$ 0.5
1,000	—	—	—	0.6	2.4	7.7	2.5	0.16
2,500	—	—	—	0.6	4.4	12.8	7	0.27

DNA was irradiated with 254 nm ultraviolet light in the presence or absence of 0.03% hydrogen peroxide. Molecular weights were determined by sedimentation in an alkaline sucrose gradient. Single strand chain breaks were estimated assuming $M_n = 0.5 M_w$ (M_n , number-average; M_w , weight-average molecular weight) and $(M_n^0/M_n) - 1 = \text{No. breaks per molecule}$ (M_n^0 , initial molecular weight). Defects per base pair were calculated assuming 1,500 base pairs per 10^6 daltons. PyPy per base pair does not include $\hat{C}\hat{C}$. The intercepts (I) were determined by extrapolation of $(-\ln \theta)/t$ back to $t=0$; I for the unirradiated sample was $1.5 \times 10^{-3} \text{ min}^{-1}$, which value was subtracted from all the intercepts to give the values shown. In addition, the intercepts for the peroxide treated samples were corrected for the small contributions estimated to come from dimers produced during irradiation.

chain breaks in the DNA¹¹. Photoproduct yields and single strand chain breaks were determined as described previously¹². The rate of reaction with formaldehyde (2.53%) was measured at 255 nm and at 54° C with a Beckman DU equipped with a Gilford model 2000 spectrophotometer attachment. The initial absorbance (A_{initial}) of the solutions was measured immediately after adding formaldehyde. After 90 min at 54° C, the temperature was raised to 90° C for 10 min and the final absorbance (A_{final}) was measured. The absorbance data were treated as described by Trifonov *et al.*⁴ and Lazurkin *et al.*⁵ to obtain I , the initial rate of unwinding.

To convert pyrimidine dimers into chain breaks, the ultraviolet-endonuclease isolated from *Micrococcus luteus* (a gift from W. L. Carrier) was allowed to react with the irradiated DNA for 30 min at 37° C. The enzyme was removed from the DNA before the formaldehyde reaction by extraction with an equal amount of 24:1 chloroform:alcohol. The DNA was then dialysed, with three changes, for 24 h against 0.01 M phosphate buffer.

The rate of formaldehyde reaction with DNA irradiated to form dimers or photolysed with hydrogen peroxide to make chain breaks was determined, and the rates of unwinding, I , were obtained. A value of ν , the velocity of unwinding per defect, was then calculated from the known concentration of defects (either chain breaks or dimers). As Table 1 shows, the average value of ν for breaks was 2.2 min^{-1} which agrees well with the value 2.4 to 2.5 min^{-1} obtained by Trifonov *et al.*⁴ for sheared DNA. This value is about an order of magnitude greater than that obtained for dimers, as indicated in Table 1. The difference does not, however, rule out the possibility proposed by Shafranovskaya *et al.*⁶ that the lower velocity of unwinding for dimers reflects clustering of dimers in the irradiated DNA.

To test their hypothesis we first treated irradiated DNA with ultraviolet-endonuclease, to convert dimer sites into chain breaks, and then treated it with formaldehyde. As Fig. 1 shows, the enzyme-treated sample reacted considerably faster

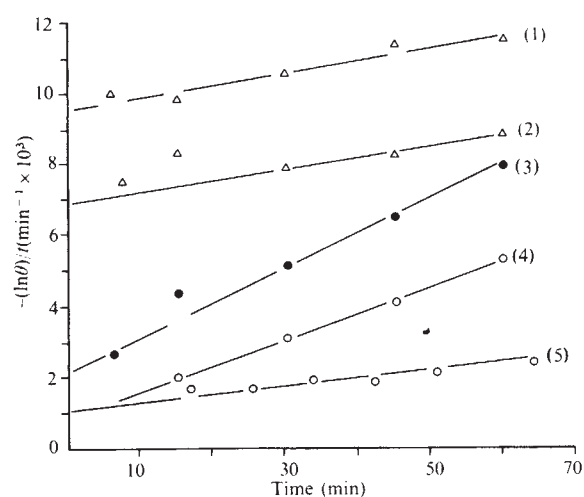


Fig. 1 Decrease in helicity, plotted as $(-\ln \theta)/t$ of ultraviolet-irradiated DNA exposed to formaldehyde for increasing lengths of time. Samples were treated with endonuclease in order to convert pyrimidine dimer sites into chain breaks. Quantitative analysis of the data is presented in Table 2. Curve (1) 800 J m⁻² with endonuclease; (2) 400 J m⁻² with endonuclease; (3) 800 J m⁻² without endonuclease; (4) no ultraviolet, with endonuclease; (5) no ultraviolet, no endonuclease.

than the untreated sample as judged by the large difference in their intercepts at $T=0$. (Enzyme treatment alone did not affect the intercept, although there was an increase in the slope of the curve.) As Table 2 indicates, the number of breaks per base pair made by the enzyme treatment was 3.3×10^{-3} , as compared with the initial concentration of pyrimidine dimers, 3.1×10^{-3} . This result implies that enzyme treatment made a break at the site of every dimer. It is calculated from the intercepts that the velocity of unwinding at chain break defects is seven to eight times faster than at pyrimidine dimer sites. This

Table 2 Kinetic Formaldehyde Analysis of Irradiated DNA Before and After Treatment with Ultraviolet Endonuclease

Ultraviolet fluence (J m ⁻²)	Endonuclease	M_w (daltons $\times 10^{-6}$)	Breaks per base pair ($\times 10^3$)	$\hat{U}T/T$ ($\times 10^3$)	$\hat{T}T/T$ ($\times 10^3$)	PyPy per base pair ($\times 10^3$)	I (min ⁻¹ $\times 10^3$)
400	—	4.39	—	4.8	7.6	3.1	<1
800	—	—	—	—	—	~6*	1.2
0	+	4.74	0	0	0	0	0
400	+	~0.36	3.4	4.1	8.1	3.1	5.5
800	+	—	~6*	—	—	—	8.7

* Since the samples receiving 800 J m⁻² contained no radioactive label, these values are estimations based on extrapolations from this and previous work.

difference suggests strongly that the result reported by Shafranovskaya *et al.*⁶ is not due to clustering.

Our conclusion—that chain breaks and dimers react with different velocities—is not surprising as the structure of DNA in the vicinity of a defect containing a single strand break would be expected to be less organised than that around a site containing a dimer, at which the sugar-phosphate backbone is still intact. Hence, formaldehyde reaction would be more likely to occur at a chain break than at a dimer.

A similar conclusion regarding base damage caused by ionising radiation was reached by Poverennyi *et al.*¹³, who observed that gamma-irradiated DNA reacted at the same rate with formaldehyde as DNase-treated DNA containing the same number of single strand breaks. Since gamma-irradiated DNA contains 4.5 times as many damaged bases (as determined by the loss of absorbance) as chain breaks, they concluded that base damage makes a negligible contribution to the reaction of gamma-irradiated DNA with formaldehyde.

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Effects of Low-dose X-irradiation on Chromosomal Non-disjunction in Aged Mice

THE factors responsible for the increasing incidence of offspring with Down's syndrome in older women are a continuing subject of debate. The demonstration of a greater frequency of aneuploid embryos in old mice^{1,2} may open the way for further experimental studies of the causes of some types of chromosomally abnormal conceptuses.

Clinical evidence suggests that the effect of maternal X-irradiation on the incidence of mongoloid children is a relatively small one³. Yamamoto *et al.*⁴ have presented results which, they claim, demonstrate that the bivalents of aged mouse oocytes are more susceptible to environmental injury than those of young oocytes. There is a decline in chiasma frequency and a corresponding increase in univalent frequency with age in mouse oocytes at diakinesis^{5,6}. Yamamoto *et al.*⁴ suggest that a deficiency of chiasmata could have predisposed aged oocytes to chromosomal damage during the long dictyate stage of meiosis. They argue from their results that, whereas

there was no significant effect of low-dose X-irradiation on the ova of young mice, there were significantly more chromosomally abnormal ova produced in old mice compared to young mice after irradiation. They emphasise the further increase in aneuploidy in old treated mice compared to old controls ($P=0.0337$ and 0.0600 for one and two-tailed tests, respectively). From all this they deduce (wrongly in our opinion) that the age of the mouse significantly affects the susceptibility of oocytes to irradiation damage. It should perhaps be mentioned that in comparing such small incidences, relatively large samples are required if significant differences are to be detected.

Table 1 Effect of low-dose X-irradiation on chromosome complement of 10.5-d-old mouse foetuses

Group	Total No. foetuses	No. aneuploid foetuses	% aneuploid foetuses (P)
Young non-irradiated	149	2	1.34
Young irradiated	111	4	3.60
Old non-irradiated	156	10	6.41
Old irradiated	43	7	16.28

* From the results of Yamamoto *et al.*⁴.

We have re-examined the data of Yamamoto *et al.*⁴ (Table 1) for evidence of interaction between age and X-irradiation. The differential effect of irradiation on ova from young and old mice was tested by comparing the appropriate function of the percentages with its standard error⁷. The value obtained was $(16.28 - 6.41 - 3.60 + 1.34) = 7.61$, with a standard error of 6.29, certainly a non-significant deviation from zero. This finding was confirmed by partitioning the three degrees of freedom of the contingency table into an age, irradiation and interactive effect. The latter comparison was found to be non-significant.

Both these statistical procedures, aimed at testing the conclusions of Yamamoto *et al.*, use linear differences in the percentages, which at such low incidences could prove quite misleading. A more meaningful method would be to examine the proportionate increases apparently due to irradiation, resulting in this example in the very similar ratios of $16.28/6.41 = 2.54$, and $3.60/1.34 = 2.69$ for old and young mice respectively. We have concluded therefore that these results do not show evidence of an interrelation of maternal age and radiation treatment.

Aneuploid embryos found in this study included uniform monosomics and trisomics as well as mosaics. Uniform aneuploids probably arise through chromosomal non-disjunction at meiosis I or, less likely, meiosis II, whereas mosaicism is produced by two cell lines developing in a single zygote. In the analysis of Yamamoto *et al.*⁴ these anomalies were grouped together, a fact which makes their conclusions even more questionable. If the mosaic embryos are excluded the incidence of aneuploid embryos found in their data is still higher in old (2.5%) compared to young (0) non-irradiated females ($P=0.0672$). A closer examination of the results of Yamamoto *et al.*⁴ strongly suggests that the data from their earlier published results¹ have been used as controls for the current irradiation studies. Such a procedure seems highly questionable in view of possible differences in environmental conditions.

In view of these objections we consider that the differential effect of X-irradiation on young and old oocytes to be not proven.

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New selective Giemsa technique for human chromosomes, C_a staining

AFTER the introduction of the quinacrine fluorescence method¹, several Giemsa staining techniques have been developed for karyotype analysis of human chromosomes. Pardue and Gall², originally noticed a denser staining of centromeric regions of chromosomes after *in situ* hybridisation of mouse chromosome preparations with mouse satellite DNA followed by Giemsa staining. This initial approach was modified by Arrighi and Hsu³ who omitted DNA hybridisation. Later, Sumner *et al.*⁴ left out treatment with RNase and HCl as well. Finally, McKenzie and Lubs⁵ treated chromosomes with HCl and 2×SSC only. These modified Giemsa procedures all produce densely stained regions of one or both chromosome arms close to the centromere. These C-band procedures also stain the secondary constriction of chromosome 1, 9 and 16, as well as the distal part of the Y chromosome. Satellites that stain brightly by Q-band techniques are also revealed by these methods.

Recent improvements of staining procedures have allowed an identification of particular chromosome areas. Among others Gagné and Laberge⁷ and Bobrow *et al.*⁸ have developed different techniques that selectively stain the secondary constriction of chromosome number 9.

Here I report a new banding technique which reveals two identical dots at the place of the centromere, one on each chromatid. The dots appear to have the same size on all forty-six human chromosomes (Fig. 1). This pattern may be termed C_a, for centromeric dots. The dots give the appearance of very densely stained and sharply delineated spheres. The

uniform size of the dots may suggest that they represent organelles associated with the spindle fibres. Prometaphase chromosomes tend to show a very narrow band instead of the separate dots, perhaps indicating that the centromere has not yet divided at this stage. The technique is specific for the centromeric regions, in contrast to other C-band treatments, that also give staining of the secondary constriction of chromosome 1, 9 and 16, the distal part of the Y chromosome, as well as satellites.

The procedure was as follows: human metaphase preparations were obtained by culturing heparinised whole blood, 0.2 ml per 3 ml 30% human inactivated AB-serum in Earle (MEM) medium. The culture was collected after 72 h and treated with colcemid (in a final concentration of 0.2 µg ml⁻¹) during the last 2 h. Hypotonic treatment followed, with 0.075 M KCl (5 min) at room temperature. The cells were fixed three times in methanol: acetic acid. The first time, the ratio of methanol: acetic acid was 9:1; the second time, 5:1, and the third time, 3:1. The cells were spread directly by a Carlsberg pipette on a wet glass surface at 13° C and air dried. Slides which had been stored for 1 week at room temperature were incubated in Earle's BSS medium (pH 8.5–9.0) at 85° C for 45 min. The slides were then stained in 1/25 Giemsa (Merck) in a 1/300 M buffered phosphate (pH 6.5) for 10 min. It seems very important to standardise the production procedure for chromosome preparation before heat treatment for C_a bands to be produced routinely. The culturing, hypotonic, and fixation treatment, as well as the age of the slide after preparation, do influence the banding pattern. The same parameters have also been found important in the case of the G, C and R-banding techniques⁹.

The physicochemical basis of this staining reaction is not yet understood. But it is known that the highly repetitive DNA found in areas of constitutive heterochromatin can be divided into various DNA families, differing in base composition, repetitiveness, and so on¹⁰. C_a bands possibly represent a specific DNA-protein complex which has been preserved after heat and alkali treatment.

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Relationship between DNA content of polytene chromosome bands and heterochromatization in *Drosophila*

THE variegated position effect is associated with chromosomal rearrangements involving heterochromatin (reviewed in ref. 1). In *Drosophila melanogaster*, cytological studies of the salivary gland chromosomes of strains carrying such rearrangements show that in some cells, the chromosome regions adjacent to broken heterochromatin lose their char-

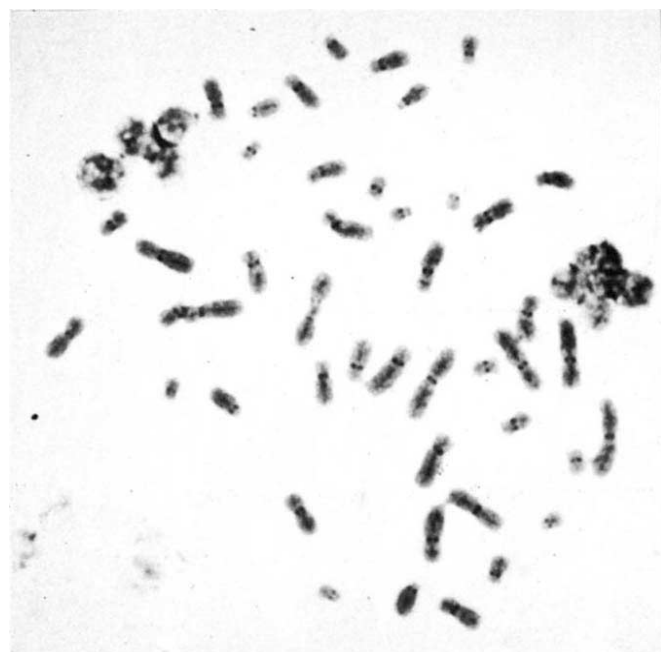


FIG. 1 Human metaphase chromosomes (46, XY). C_a bands. Only the centromeric regions are stained. They contain two identical dots at the centromere, one on each chromatid.

TABLE 1 Feulgen-DNA contents of chromosome regions in two strains of *D. melanogaster*

Region(s)	Single inversion strain*	Double inversion strain*	Diff. of means $\pm$ s.e.	P
	$M \pm$ s.e.	$M \pm$ s.e.		
7E1-2	55.816 $\pm$ 5.594	58.075 $\pm$ 1.984	2.259 $\pm$ 5.935	n.s.
10A1-2	51.991 $\pm$ 6.703	53.625 $\pm$ 1.796	1.634 $\pm$ 6.939	n.s.
10D1-2	28.741 $\pm$ 3.020	23.941 $\pm$ 2.124	4.800 $\pm$ 3.692	n.s.
	$\bar{R} \pm$ s.e.	$\bar{R} \pm$ s.e.	$t_{22}^\dagger$	P
10D/7E	0.525 $\pm$ 0.032	0.414 $\pm$ 0.034	3.476	<0.01
10D/10A	0.574 $\pm$ 0.037	0.449 $\pm$ 0.040	2.315	<0.05
10A/7E	0.925 $\pm$ 0.044	0.927 $\pm$ 0.025	0.040	n.s.
	r	r	t ‡	P
10D and 7E	0.814	0.297	1.805	n.s.
10D and 10A	0.874	0.269	2.332	<0.05
10A and 7E	0.877	0.660	1.138	n.s.

Salivary gland squashes from female white prepupae cultured at 14°C were stained by the Feulgen method. Using a Vickers M85 scanning microdensitometer, the integrated absorbance at 570 nm of the selected chromosome regions was measured in six non-heterochromatised nuclei in each of four preparations, two from each genotype. A $\times 100$ Planapochromatic objective of NA 1.3 gave a flying spot of diameter 0.47 μ m in the specimen plane, and 4% glare was offset electronically. Measured areas were precisely demarcated with a variable rectangular masking frame, and the apparent absorbance of an identical area of background, set to 90% transmission, was subtracted from each experimental reading. Each region was measured six times and the mean recorded.

M : mean Feulgen-DNA content of region in relative units.

$\bar{R}$: mean of ratios obtained by dividing Feulgen-DNA contents of region by that of reference band in same nucleus. r : correlation coefficient of Feulgen-DNA contents of two regions. For each strain, $n = 12$.

* for genotypes, see text.

$^\dagger t(22)(0.05) = 2.074$

$^\ddagger t(\infty)(0.05) = 1.960$

acteristic banded appearance and become heterochromatised. The extent of this morphological change varies from cell to cell, but it has been noted that heterochromatisation seems to terminate preferentially just short of relatively large deeply staining bands^{2,3}. This observation might be due to the fact that such bands are more readily visible, and hence more likely to be scored as euchromatin than smaller bands. The involvement of other factors is however suggested by the observation³ that heterochromatisation associated with a given inversion in two genetic backgrounds terminates with different frequencies adjacent to certain bands which were morphologically similar in the two strains. A factor which, it has been suggested⁴, may affect the progress of heterochromatisation is a prior change in the nucleic acid content of affected regions. Early measurements of nucleic acid content using ultraviolet photographic microdensitometry of whole bands⁴ or spot estimations of optical density⁵ yielded equivocal results. We decided to use scanning microdensitometry to investigate the possibility that the differences in extent of heterochromatisation observed in our strains might be related to the DNA content of the bands concerned.

The Feulgen-DNA content of one affected band, 10D1-2, (terminology according to Bridges⁶) was measured in the single and double inversion strains, m^k ;+ and m^k ;Rev^b. To correct for variations in staining and polyteny, the Feulgen-DNA contents of bands 10A1-2 and 7E1-2 were used as internal standards: 10A1-2 lies within the region subject to position effect but shows the same response to heterochromatisation in both strains, and 7E1-2 is beyond the region of potential heterochromatisation.

The results are summarised in Table 1. Mean uncorrected Feulgen-DNA contents of bands did not differ significantly between the strains, although in the case of band 10D1-2 the decrease in the double inversion strain approached statistical significance. When standardised against either of the reference bands, the mean value for 10D1-2 was significantly smaller in the double than in the single inversion strain. These results were confirmed by analysis of covariance (10D1-2 and 7E1-2: $F(1/22) = 4.55$, $P < 0.05$; 10D1-2 and 10A1-2: $F(1/22) = 4.63$, $P < 0.05$). In the single inversion strain there was a significant correlation between the Feulgen-DNA contents of 10D1-2 and each reference band, while in both strains there was significant correlation

between the two reference bands. Thus in the double inversion strain band 10D1-2 showed a relative, and possibly an absolute, decrease in Feulgen-DNA content and also exhibited less related variation than in the single inversion strain. Measurements were also made of band 50A1-2, a euchromatic band within the Rev^b inversion; of band 80A1-2, a relatively well defined heterochromatic band at the base of chromosome 3L; and of part of the chromocentral region which corresponded morphologically as closely as possible in each nucleus. No strain differences were found at these sites.

Band 10D1-2 therefore seems to be unusual not only in its relation to termination of heterochromatisation but also in its Feulgen-DNA content. The coincidence of these peculiarities suggests that the apparent irregularity in progression of heterochromatisation along the chromosome is not merely an observational artefact, but rather a genuine phenomenon related to some biological property of the bands concerned. Since the DNA content of a prepupal chromosome band presumably reflects the replication behaviour of the band during embryonic and larval development, a causal connection between heterochromatisation and DNA replication seems plausible. It should be noted that the chromosomes selected for measurement were not heterochromatised. Our results indicate that an additional rearrangement not only affects variegation and heterochromatisation³, but also influences chromosome bands in (potentially heterochromatised) regions which are morphologically euchromatic.

It is tempting to speculate that a break in heterochromatin leads to a change in replication behaviour of some euchromatic bands in its vicinity, and that the progression of heterochromatisation, when it occurs, is affected by such changes.

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Light-regulated guanosine 3', 5'-monophosphate phosphodiesterase of bovine retina

RETINAL rod outer segment (ROS) adenylyl cyclase activity seems to be light sensitive, implicating adenosine 3',5'-monophosphate (cyclic AMP) in photoreceptor function^{1,2}. Guanosine 3',5'-monophosphate (cyclic GMP) could also play a role in photoreceptor function since the synthetic (guanylyl cyclase)³ and degradative (cyclic GMP phosphodiesterase)⁴ enzymes are highly active in purified ROS. In particular the specific activity of guanylyl cyclase is higher in bovine photoreceptor structures than in any other tissue studied. This activity, however, is not influenced by illumination³.

To assess cyclic GMP formation in a more physiological system, we followed the formation of ³H-cyclic GMP from ³H-hypoxanthine in isolated intact cattle retina. In the presence of a phosphodiesterase inhibitor, no differences could be detected between illuminated and dark-adapted retinae. By contrast, without the inhibitor, a much lower ³H-incorporation was observed after preillumination (Table 1).

TABLE 1 Formation of ³H-guanosine 3',5'-monophosphate from ³H-hypoxanthine by bovine retina

	c.p.m.g ⁻¹ retina	
	With	Without
Exp. 1	SQ 20,009	SQ 20,009
Dark	4,040	3,490
Light	3,420	1,000
Exp. 2		
Dark	6,520	4,580
Light	6,280	740

Young bovine eyes were obtained from a slaughterhouse and dark-adapted for 2 h at 0° C. Subsequent operations were carried out in dim red light. The retinae were preincubated for 30 min at 37° C in Krebs-Ringer-bicarbonate buffer (pH 7.4, continuously gassed with 95% O₂ - 5% CO₂). After prelabelling (60 min at 37° C) with 2.5 μM ³H-hypoxanthine (5 μCi per retina), the retinae were washed by five successive transfers into 100 ml beakers and then distributed one by one into 10 ml Erlenmeyer flasks containing 3.5 ml Krebs-Ringer-bicarbonate buffer with or without 0.3 mM cyclic nucleotide phosphodiesterase inhibitor SQ 20,009 [1-ethyl-4-(isopropylidenehydrazino)-1H-pyrazolo-(3,4-b)-pyridine-5-carboxylic acid ethylester]. The retinae were then either left in the dark (0° C), or illuminated at 0° C in 5 ms (unfiltered flash light having a maximum energy of 30 J, 10 cm above the incubation vessel). The final incubation (10 min at 37° C) was started immediately. The retinae were collected by rapid filtration and homogenised in 8% trichloroacetic acid (TCA). TCA was extracted by ether and the ³H-cyclic GMP separated from other nucleotides by successive chromatography on aluminum oxide (1.5 g Al₂O₃, Woelm W200 neutral) equilibrated with 0.06 M Tris-Cl buffer, pH 7.5) and Dowex 1 × 8 (formate, 0.5 × 4.5 cm) columns. The Al₂O₃ columns were washed with 2 ml 0.06 M Tris-Cl buffer, pH 7.5, and cyclic GMP eluted with 2 ml 0.6 M Tris-Cl buffer, which was passed through the Dowex columns. After a wash with 6.5 ml 0.5 N formic acid the cyclic GMP was eluted with 3 ml 8 N formic acid. Unlabelled cyclic GMP had been added to the TCA extracts to correct for recovery (51-64%). If the Dowex eluate was treated with beef heart phosphodiesterase (Sigma), less than 5 % of the radioactivity originally present could be detected after a subsequent chromatography on Dowex 1 × 8. No radioactivity from added ¹⁴C-cyclic AMP was found in the cyclic GMP fraction.

The results are expressed as c.p.m. in ³H-cyclic GMP per g fresh weight. Values are the mean for two retinae incubated in parallel, the single determinations differed from the mean less than ±12 %.

Although these values, uncorrected for the specific activity of GTP, by no means measured exactly the synthesis of cyclic GMP, they indicate that retinal cyclic GMP levels could be regulated by light-dependent changes in phosphodiesterase activity. In fact, the cyclic GMP phosphodiesterase activity of retinal homogenates incubated in the light was more than three times that of homogenates assayed in the dark (Table 2). But in accord with previous studies,¹ cyclic nucleotide phosphodiesterase activity of purified ROS was not light sensitive. As expected, illumination was without effect on brain phosphodiesterase.

These results might suggest that a phosphodiesterase localised elsewhere than in the ROS could be influenced by light. But 84% of total retinal cyclic GMP hydrolysing activity is associated with the outer segments prepared and tested in the dark (unpublished).

With cyclic AMP as substrate, a much smaller light activation was seen (Table 2). This result is in keeping with the observation that cyclic GMP is the preferred substrate for ROS cyclic nucleotide phosphodiesterase, whereas an activity with a low *K_m* for cyclic AMP is localised in other retinal structures^{4,6}.

TABLE 2 Effect of light on retina cyclic nucleotide phosphodiesterase

Preparation	nmol GMP formed/mg protein/min	
	Light	Dark
Bovine retina homogenate	29.0 ± 1.6*	8.5 ± 0.8*
Bovine rod outer segments	157	154
Rat brain homogenate	28.3	26.7
	nmol AMP formed/mg protein/min	
	Light	Dark
Bovine retina homogenate	9.3 ± 0.6†	6.3 ± 0.8†

Retinae from dark-adapted young bovine eyes (1-3 h at 0° C) were homogenised by hand in 5 volumes (w/v) 0.25 M sucrose-10 mM Tris-HCl buffer, pH 7.5, and aliquots (600-800 μg protein) assayed⁵ for cyclic nucleotide phosphodiesterase activity with either 1 mM cyclic GMP or 1 mM cyclic AMP in diffuse room light or in complete darkness (5 min at 37° C). To exclude the possibility that the reaction might go further than to the corresponding nucleosides, ³H-GMP and ³H-AMP were incubated with retina homogenates. Their recoveries were not influenced by light and virtually identical with those of the ³H-nucleosides added at the end of the incubation (70-76% for guanosine and GMP, 64-66% for adenosine and AMP).

* Mean value for nine experiments ± s.e.m. (*P* < 0.01).

† Mean value for four experiments ± s.e.m. (0.05 > *P* > 0.01).

The light activation could be mimicked by adding bleached ROS preparation devoid of phosphodiesterase activity and shown to contain no protein other than opsin, by gel electrophoresis⁷. ROS, prepared as described elsewhere⁸, were extracted with 0.1% sodium dodecyl sulphate (SDS), which was removed by repeated washing. Such preparations, left in the dark or illuminated until 65% bleaching had occurred, were added to the cyclic GMP phosphodiesterase assay. There was no effect on retina phosphodiesterase activity determined in the light (Table 3). Addition of the bleached preparation, however, increased the dark activity almost up to the activity determined in the light. Unbleached rhodopsin had also a small stimulating effect on the dark activity, possibly due to the presence of some bleached rhodopsin. The rhodopsin preparations contain membrane-bound lipids including retinal. But all-trans retinal, which is released during bleaching, had no effect on cyclic GMP phosphodiesterase activity measured in light or dark if added in concentrations equivalent to the amount present in the rhodopsin preparations. Traces of bound SDS could have a stimulatory effect. The SDS should, however, exert its effect on both light and dark activity. Furthermore, brain and liver phosphodies-

TABLE 3 Effect of adding preparations of photoreceptor membranes on retinal cyclic GMP phosphodiesterase activity

Addition	nmol GMP formed/mg protein/min	
	Light	Dark
Dark-adapted ROS preparation	33.9	14.5
Bleached ROS preparation	33.8	32.6
Control	34.3	8.7

ROS purified as described previously,⁸ were extracted twice with 0.1 % SDS—10 mM Tris-HCl buffer (pH 7.5). The insoluble material was washed first six times with 0.25 M sucrose—10 mM Tris-HCl (pH 7.5) and finally taken up in the same buffer (1 mg protein ml⁻¹). One part of the preparation was illuminated for 5 min at 0° C (33 W tungsten lamp, distance 4 cm), the rest left in the dark. Spectrophotometric estimations⁹ showed that 65% of the rhodopsin was bleached in the light-exposed preparation and less than 10% in the dark-adapted one. Aliquots containing 30 µg protein were added to samples of a retina homogenate (650 µg protein) and cyclic GMP phosphodiesterase activity determined in diffuse room light or in complete darkness. Buffer only was added to the controls.

terases, though stimulated by low SDS, were affected neither by dark- nor by light-adapted preparations.

Cyclic nucleotide phosphodiesterase inhibitors have been shown to have a profound effect on frog¹⁰ and *Limulus*¹ photoreceptor potentials. Our data show that regulation of ROS phosphodiesterase activities could be also physiologically important, since retinal cyclic GMP phosphodiesterase activity was strongly influenced by light. The light effect was most probably dependent on the bleaching state of rhodopsin, since it could be mimicked by adding light-exposed ROS preparations devoid of phosphodiesterase activity, where all components soluble in buffer and low concentrations of SDS had been eliminated. The light-provoked decrease of ³H-cyclic GMP levels in incubated retinæ, not seen after phosphodiesterase inhibition, suggests that light-dependent changes in cyclic GMP phosphodiesterase activity occur also in intact retinæ.

Cyclic nucleotide phosphodiesterase of taste receptors has been shown to be activated by bitter taste stimuli¹¹. Activation of phosphodiesterase activity by the appropriate stimuli might be a general phenomenon for the function of sensory receptors.

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Correlations between (Na + K)-ATPase, Ca-ATPase and cellular potassium concentration in cattle red cells

THE existence in erythrocytes of active, ATP-dependent Na-K exchange on one hand and of active, ATP-dependent outward transport of Ca²⁺ on the other raises the question whether two independent or one common system account for these functions. Inhibition of the Na-K system by Ca, although easily demonstrated, does not lend particularly strong support to the hypothesis of a common pathway for Na and Ca in active transport, because full activation of the Ca system⁶⁻⁹ is achieved by lower Ca concentrations than those required to inhibit Na+K-ATPase⁸ and because Epstein and Whittam¹⁰ have pointed out that the effect is due to competition between Ca-ATP and Mg-ATP for the substrate site and that competition between Ca and Na for the transport site is unlikely. The Na-K system is inhibited by cardiac glycosides whereas Ca transport and Ca-activated ATPase are not^{2,3,11} which seems to exclude a common site, but not necessarily a common protein. If alkali cation and Ca movements were mediated by the same membrane protein independent measurements of Na+K-activated ATPase and Ca-stimulated ATPase activity should positively correlate. K concentration in cattle red cells varies widely between animals^{12,15}, there is agreement as to the ability of these cells to pump Na and K¹³⁻¹⁷, and indications exist that different intensity of pumping accounts for the different K (and Na) content of the cells¹²⁻¹⁴. Cattle cells, therefore, seem suitable objects to study phenomena which might correlate with Na+K-ATPase.

Blood (100 ml) from healthy adult (above 2-yr-old) Simmental and Swiss Brown cows was prevented from clotting by adding approximately 17 U ml⁻¹ heparin (USP) and brought to the laboratory within a few hours. The cells were washed four times with a solution of (mM) 120 choline-Cl, 40 Tris-Cl, pH 7.4 in the cold. Na-free solution was chosen to avoid any possible Na-Ca exchange during washing. White cells were discarded. K in washed and packed cells was measured by flame photometry in the haemolysate (0.1 ml cells + 7.9 ml H₂O). For the measurement of cellular Ca the cells were diluted 1:4 with water, the mixture deproteinised with an equal volume of trichloroacetic acid (10%) and the supernatant made up to 50 mM LaCl₃ in another 1:2 dilution step to prevent interference of phosphate. Standards of CaCl₂ were made up in the same water and LaCl₃ solution and the samples were measured with an EEL atomic absorption flame photometer with scale expansion unit and recorder. Results were corrected for Ca contamination of trichloroacetic acid. The choline-washed cells were further washed twice with a solution of (mM) 150 NaCl, 5 KCl, 1 MgCl₂ and membranes were prepared from them according to Wolf⁷ as described earlier⁶. The membranes were kept frozen until used (36 h at most). Na+K-ATPase was measured as the ouabain-inhibitable fraction in a medium of (mM) 100 NaCl, 10 KCl, 4 MgCl₂, 0.5 tris-EGTA, 30 imidazole-Cl, pH 7.0 (at 37°C), 2 Mg-ATP, with or without ouabain 10⁻⁴ g ml⁻¹. Ca-ATPase was assayed in the following medium: (mM) 70 choline-Cl, 4 MgCl₂, 30 imidazole-Cl, pH 7.0 (at 37°C), 0.5 Ca-EGTA buffer (of 10⁻⁵M Ca²⁺ concentration) or 0.5 Tris-EGTA, 2 Mg-ATP and 10⁻⁴ g ml⁻¹ ouabain. The samples contained on average 1.47 mg protein in 2.5 ml, incubation time was 90 min and temperature 37°C. In six experiments Ca-ATPase was measured in the absence of Mg, Mg-ATP being replaced by Na-ATP. All samples were run in duplicates. P_i liberated and protein were measured as before⁶. Since high K cells are rare^{12,13} many animals were sampled for K content of cells and twenty-one specimens were selected such as to have all K concentrations about equally represented.

It was found that cellular Ca concentration is far below the Ca^{2+} concentration in blood plasma, much as was reported for human red cells^{18,19}. The average from thirteen

animals was $0.024 \pm 0.003 \text{ mmol l}^{-1}$ cells ($\pm$ s.e.m.). This is not due to the washing procedure since unwashed cells gave values similar to what was predicted on the assumption of virtually Ca free cells contaminated by 6–8% blood plasma. No correlation between cellular Ca content and Ca-ATPase activity of the corresponding membranes was found, which is not surprising, as it is well known that in human red cells total Ca is largely accounted for by Ca associated with the membrane and does not reflect intracellular Ca concentration^{18,19}.

Figure 1a shows the frequency distribution of the Ca-ATPase in twenty-one animals. The average, omitting the three outliers, was $0.0151 \pm 0.0024 \text{ } \mu\text{mol per mg protein per h}$ ($\pm$ s.e.m.) which is about 1/50 of what is found in human red cells under similar conditions. The outliers were 9.6 standard deviations or more away from the mean and were, therefore, omitted from the statistical evaluation or replaced by the highest value of the rest of the distribution (0.0348) as shown in Fig. 1 by solid symbols (C. P. Windsor, quoted in ref. 20.) Preliminary experiments indicated that the dissociation constant K_d of the enzyme for Ca^{2+} is of the order of 10^{-6}M . There is some Ca-stimulated activity in the absence of Mg. In six experiments this amounted to 14.3% of the total Ca-stimulated activity in the presence of Mg. This activity was not separated from the total in the statistical treatment.

Figure 1b shows that a close positive correlation exists between Na+K-ATPase activity and cellular K content. The calculated regression line extrapolates to the reasonable K concentration of 6 mmol l^{-1} cells at zero Na+K-ATPase activity. This is according to expectation and agrees with the accepted view that the Na-K pump is a major factor controlling cellular K concentration.

The main finding is illustrated in Fig. 1c. Ca-ATPase activity and Na+ATPase activity correlate negatively ($P < 0.05$ when the outliers for Ca-ATPase are omitted, $P < 0.01$ when they are reduced to $0.0348 \text{ } \mu\text{mol mg}^{-1} \text{ h}^{-1}$ in a two-tailed test). Triangles represent samples which can be attributed with a high degree of probability to the class of animals with genetically determined high K cells in our breeds^{12,13}. Within this group the correlation coefficient (r) is -0.807 (with $P = 0.009$) when the outlier is reduced to 0.0348.

Figure 1d demonstrates that no significant correlation was found between Ca-ATPase activity and cellular K content. But when the partial correlation coefficient ($r_{D.bc}$) is calculated (eliminating the effect of the correlations b and c of Fig. 1), a positive correlation is revealed with $r_{D.bc} = 0.450$ and $z \sqrt{N-4} = 1.953$. For a two-tailed test

$$z \sqrt{N-4} = \frac{1}{2} \cdot \ln[(1 + r_{D.bc})/(1 - r_{D.bc})] \cdot \sqrt{N-4} \\ > 1.96 \text{ for } P < 0.05$$

with N = number of points. This means that the positive correlation is just significant. Thus it seems not unlikely that the negative correlation between Na+K-ATPase and Ca-ATPase masks a positive correlation between Ca-ATPase and K content of the cells.

The negative correlation between Ca-ATPase and Na+K-ATPase makes the hypothesis of a single system for both improbable. Competition between alkali cations and Ca can be ruled out because Na+K-ATPase was assayed in the absence of Ca and Ca-ATPase was measured in the presence of Na and K (except for approximately 0.2 mM stemming from the membrane preparation) and in the presence of ouabain. No interpretation with respect to cause and effect can be offered for the negative correlation at present, but several possibilities might be considered. (1) The Na-K pump requires traces of Ca inside the membrane and the Ca pump removes Ca more effectively from the critical region than EGTA alone does. (2) A fixed number of molecules of an individual protein are synthesised in the maturing cell

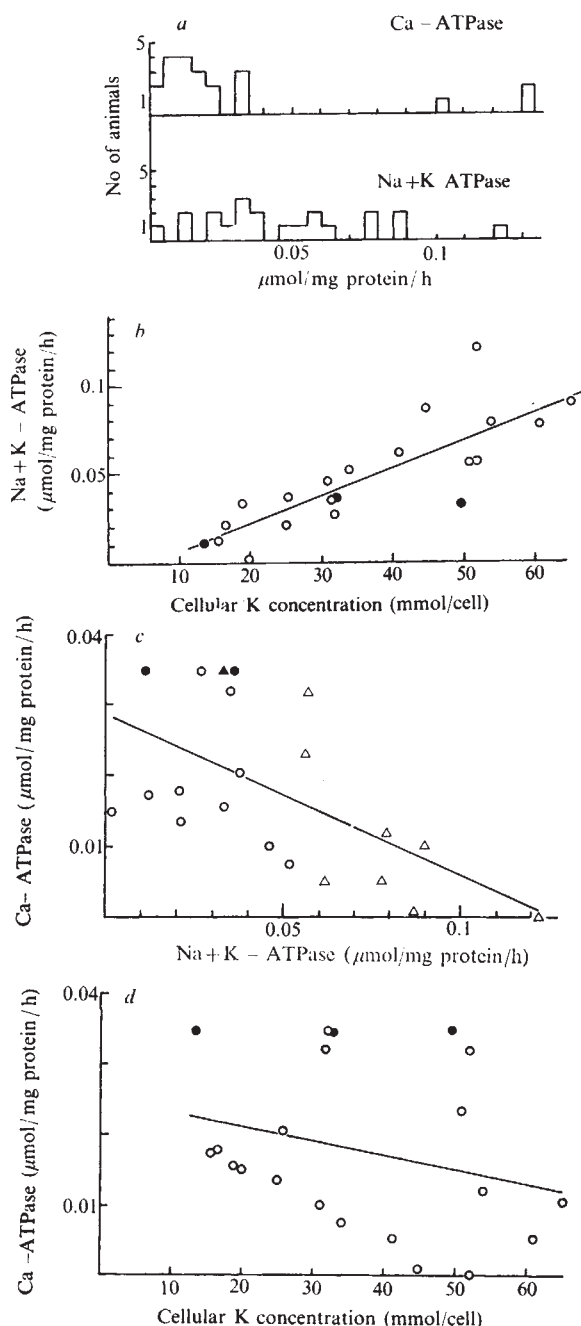


FIG. 1 Cellular K concentration, Na+K-ATPase and Ca-ATPase activity in erythrocytes of twenty-one cows. Ca-ATPase: difference between sample with 10^{-5}M Ca^{2+} and sample with 0.5 mM EGTA (medium: choline 70 , imidazole 30 , Mg-ATP 2 , Mg 4 , Cl 108 mM , ouabain 10^{-5} g/ml). Na+K-ATPase: difference between sample without and with ouabain $10^{-4} \text{ g/ml}^{-1}$ (medium: Na 100 , K 10 , imidazole 30 , Mg-ATP 2 , Mg 4 , Cl 148 mM). pH 7.0 , 37°C , 90 min . Cellular K concentration measured in fresh cells, washed with choline-Cl 120 mM , tris-Cl 40 mM , pH 7.4 . a, b, c, d: Same data plotted differently. a: Frequency distribution of Na+K-ATPase does not peak, because animals were selected in order to have all K concentrations equally represented (compare with b). Notice three outliers in the Ca-ATPase values from the same animals. b, c, d: Each point represents 1 animal. b, $r = 0.819$, $P < 0.001$; c, $r = -0.588$, $P < 0.01$; d, $r = -0.272$, $P > 0.05$. Solid symbols = Ca-ATPase outliers (Ca-ATPase values corrected as described in text). c, Triangles = animals with K concentration in cells $> 40 \text{ mmol l}^{-1}$ cells. Calculated regression lines and r values include all points shown.

and an unknown factor determines whether any one of these behaves as a Ca- or Na-K-pump site. (3) In the synthesis of two distinct pump proteins some step is common for both and limiting and the final assembly into structurally different complexes determines the functional use of the common part. (4) An unknown factor is inhibitory for one and stimulatory for the other of two distinct pumps.

The positive correlation between Ca-ATPase and K content of fresh cells might mean that a low intra-cellular Ca concentration facilitates maintenance of high intracellular K concentration. It is well known^{5,21-25} that a small increase in cellular Ca concentration renders human red cells leaky to K. The present finding might reflect this effect of intracellular Ca* and, therefore, could substantiate the view expressed by Gardos²¹ as early as 1958 that cellular Ca content might influence cellular K concentration not only under abnormal experimental conditions but also *in vivo*.

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* Note added in proof: This interpretation is doubtful, as a recent report (Jenkins and Lew, *J. Physiol., Lond.*, **234**, 41 1973) demonstrates the absence of the Gardos-effect in ruminant red cells.

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Molecular weight of a D-glucose and L-histidine-binding protein from intestinal brush borders

WE describe here a sugar and amino acid transport protein that has been isolated from a cell for the first time. It is

a homogeneous protein subunit from the mucosal brush border of hamster jejunum that is involved in the Na⁺-dependent binding of actively transported D-glucose and L-histidine. Its molecular weight was estimated to be 59,000 by Sephadex G-75 gel filtration¹, 56,000 by sodium dodecyl sulphate-acrylamide gel electrophoresis^{2,3}, and 50,934 by sedimentation velocity analysis^{4,5}. The presence of both D-glucose and L-histidine-binding sites on the same protein with a molecular weight of approximately 55,000 may account for some of the observed competitive inhibitory effects between active intestinal sugar and amino acid transport that have been reported⁶.

Our work is based on the results of many studies which have indicated that active transport of sugars and amino acids by the small intestine occurs within the brush border of the mucosal cell and requires sodium ions⁷⁻¹⁰. In an attempt to describe these absorptive mechanisms, we have shown that actively transported sugars and amino acids are preferentially bound to isolated brush borders^{11,12} and that both D-glucose and L-histidine require Na⁺ to bind to separate sites in a filamentous fraction of disrupted brush borders^{13,14}. Since the brush border is a digestive, as well as an absorptive organelle¹⁵, we have postulated that its core filaments act as a conduit for the Na⁺-dependent active transport of all monosaccharides and all amino acids which are primarily obtained from the hydrolysis of disaccharides and polypeptides on the plasma membrane facing the lumen¹⁴. The purpose of the study reported here was to solubilise the brush border fraction involved in the Na⁺-dependent binding of D-glucose and L-histidine, and determine its molecular weight.

Mucosal brush border filaments from hamster jejunum were prepared^{13,14} and labelled with ¹⁴C by incubation at 37° C for 30 min in either 0.1 μM of uniformly labelled ¹⁴C-D-glucose (288 mCi mmol⁻¹) or 0.5 μM of uniformly labelled ¹⁴C-L-histidine (240 mCi mmol⁻¹) in 25 mM Na₂HPO₄-NaH₂PO₄ buffer (pH 7.2), 10 mM MgCl₂, and 6 mM dithiothreitol (DTT). After the incubation period the suspension was centrifuged and the supernatant was removed. Then the filamentous precipitate was extracted with cold acetone. Most of the radioactivity remained in the insoluble precipitate indicating that the ¹⁴C-labelled compound was not bound to the lipid-soluble portion of the filaments. The acetone powder was solubilised by incubating the ¹⁴C-labelled material, at 25° C for approximately 12 h, in 1% SDS, 25 mM sodium phosphate buffer (pH 7.2), 10 mM MgCl₂, 6 mM DTT, and the same concentration of the ¹⁴C-labelled compound that was used in the binding experiment. Figure 1 illustrates a typical elution profile of this solution obtained by Sephadex G-75 gel filtration. In the experiment illustrated, ¹⁴C-D-glucose was bound within a sharp peak absorbing at 230 nm. Though there was some absorption before and after the peak it did not obtain any bound ¹⁴C-D-glucose. Free radioactive sugar appeared in the elution volume between 450 ml and approximately 550 ml. This was confirmed by control experiments in which only free ¹⁴C-D-glucose was placed on the column. Similar results were obtained when ¹⁴C-L-histidine was used as the binding compound. Although both ¹⁴C-D-glucose and ¹⁴C-L-histidine were bound within the same peak of absorbance, more ¹⁴C-D-glucose ($4.17 \pm 0.96 \times 10^{-8}$ mmol) than ¹⁴C-L-histidine ($1.95 \pm 0.07 \times 10^{-10}$ mmol) was bound per mg of protein as determined by four and three experiments, respectively. The amount of the ¹⁴C-labelled compound bound was calculated from its measured radioactivity and specific activity. Protein was determined by the Lowry *et al.* method using bovine crystalline serum albumin as standard¹⁶.

To verify that the isolated protein is the component involved in Na⁺-dependent binding of actively transported nutrients by the hamster jejunum, the denaturing SDS adhering to it had to be removed. This was accomplished by

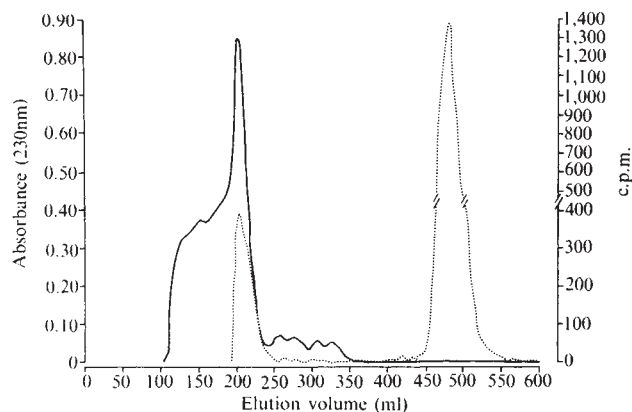


Fig. 1 Elution profile from gel filtration of acetone extracted, SDS-solubilised brush border filaments labelled with ^{14}C -D-glucose in the presence of Na^+ ions. Before the solubilised material was placed on a Sephadex G-75 gel bed in a 2.6×100 cm column, it was diluted with 25 mM of Na_2HPO_4 - NaH_2PO_4 buffer (pH 7.2) containing 10 mM MgCl_2 and 6 mM DTT to achieve a 0.1% SDS concentration, filtered with a $0.45 \mu\text{m}$ pore diameter filter, and centrifuged at $112,000 g$ for 2 h. The small amount of precipitate obtained after centrifugation was discarded. A 4 ml sample of the supernatant containing approximately 5 mg of protein were placed on the Sephadex G-75 gel filtration column eluted with 25 mM sodium phosphate buffer (pH 7.2) containing 0.1% SDS, 10 mM MgCl_2 , and 6 mM DTT. The void volume of the gel column was 140 ml. After the absorption of the eluant was read at 230 nm to detect peptide bonds, each 5 ml fraction was dried at 80°C in a vial. Then 15 ml of liquid scintillation fluid was added to each vial before it was counted in a Nuclear Chicago mark I liquid scintillation system. The peak in absorbance had bound $4.17 \pm 0.96 \times 10^{-8}$ (4) mmol of ^{14}C -D-glucose per mg of protein. An absorption spectrum of this peak was performed after it had been precipitated by 30% $(\text{NH}_4)_2\text{SO}_4$ and redissolved in 25 mM sodium phosphate buffer (pH 7.2). Absorption occurred at approximately 280 nm and 230 nm, demonstrating that the peak contained protein relatively free from other ultraviolet absorbing compounds. —, Absorbance; •••, D- ^{14}C -glucose.

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Narcotic Receptor Sites in Morphine-dependent Rats

THE biochemical basis of the development of tolerance for and dependence on narcotic analgesics has been the subject of much speculation and experiment¹⁻⁶. Theories which try to account for these closely related^{4,7} phenomena can be grouped into three main classes: (1) the body reacts to narcotic drugs by increasing the rate at which they are destroyed or neutralised (by antibodies, for example) so that a smaller proportion of the total dose reaches the brain receptors²; (2) processes which are directly affected by the narcotic analgesics are circumvented or otherwise accommodated through an indirect physiological adaptation⁶; (3) a direct adaptation takes place in which the number of receptor sites for the narcotics is greatly increased, or their affinities greatly reduced so that large amounts of drugs are needed to fill the available sites and thus effect a response⁵. We wish to present data which effectively rule out this third possibility as a major factor in narcotic drug dependence and tolerance. We show that neither the number, nor the binding affinity, nor the specificity of narcotic receptor sites⁸⁻¹⁰ is changed in the morphine-dependent (and therefore also tolerant⁷) rat brain when compared with that of the normal animal.

Osborne-Mendel rats, 150–200 g, from the NIH colony, were used, mostly males, although one set of experiments involved females, with equivalent results. Morphine dependence was induced by implanting pellets containing 75 mg of morphine base plus CM cellulose carrier and binders^{7,11}, subcutaneously near the midline of the back just behind the ears¹². Light ether anaesthesia was used. This procedure induces clear-cut tolerance to and dependence on morphine, which is maximal

dissolving the unlabelled, partially purified binding protein, obtained by precipitation with 30% $(\text{NH}_4)_2\text{SO}_4$, in 50 mM Tris-acetate buffer, pH 7.8, containing 6 mM DTT and 6 mM urea. This solution was passed through a SDS-removal column containing Dowex 1-X2¹⁷. The SDS-free effluent was then placed in the hollow bore fibres of Bio-Fiber 50 minibeaders obtained from Bio-Rad Laboratories to exchange 25 mM sodium, potassium or ammonium phosphate buffer, pH 7.2, for the Tris-acetate buffer and to remove urea. The buffer on the outside of the hollow fibres was replaced every 15 min for 1 h at 5°C . The last replacement contained $0.1 \mu\text{mol}$ of ^{14}C -D-glucose (uniformly labelled; 288 mCi mmol⁻¹). Diffusion of the ^{14}C -D-glucose across the fibre walls was then allowed to proceed for 1 h at 37°C . We had previously determined, in the absence of protein, that ^{14}C -D-glucose can reach a diffusion equilibrium under these conditions. After this incubation period, ^{14}C in 0.1 ml samples of the solutions inside and outside the hollow fibres were counted in a liquid scintillation system. The results from these studies demonstrated that ^{14}C -D-glucose was at a higher concentration within the hollow fibre compartment containing the binding protein only when Na^+ ions were present. When either K^+ or NH_4^+ were substituted for Na^+ in the phosphate buffer, ^{14}C -D-glucose was observed to be at the same concentration in both compartments. Therefore, the increase in ^{14}C -D-glucose within the protein compartment in the presence of Na^+ could only be attributed to the binding of this sugar to the isolated protein. The amount of sugar bound per mg of protein was similar to that observed in the absorbance peak from the Sephadex G-75 gel filtration column (Fig. 1).

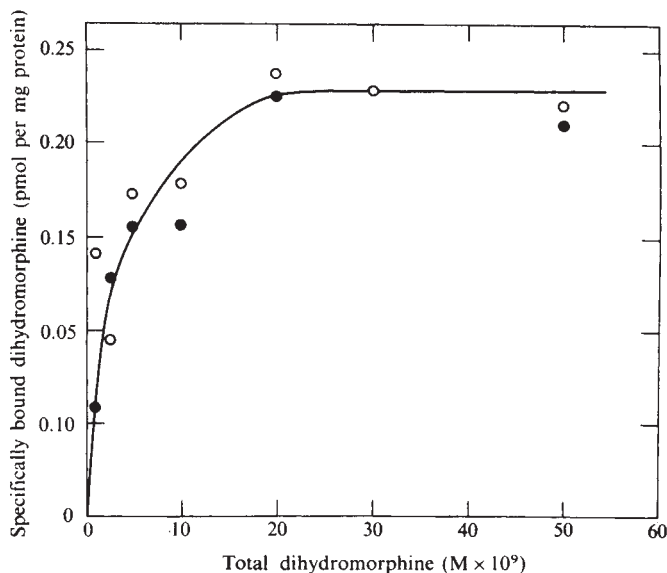


Fig. 1 The specific binding of ^3H -dihydromorphine to particulate fractions of rat brain homogenates prepared from control (○) and morphine-dependent (●) rats. The data are obtained by subtracting the amount of non-specifically bound ^3H -dihydromorphine, measured in the presence of 10^{-5} M morphine (a saturating amount) from the amount bound in the absence of unlabelled morphine. Each flask contained 0.5 mg of P_2 protein, 9 μmol Tris-HCl, pH 7.4, and ^3H -dihydromorphine (31,000 c.p.m. pmol^{-1}) in the amounts shown, in 1 ml of 0.32 M sucrose. The points shown are the average of two independent experiments each of which was performed with duplicate determinations.

after 72–96 h and declines slowly during the next few days¹². Spot checks on some animals 72 h after operation with an intraperitoneal injection of the antagonist naloxone (1 mg of the hydrochloride in 0.1 ml H_2O) showed that the animals carrying the morphine pellet went into almost immediate withdrawal^{12,13}, characterised by extreme hyperactivity, sensitivity to touch (manifest by squealing), wet dog shakes and diarrhoea. Control, sham operated, animals were unaffected by naloxone.

The animals were decapitated 72–84 h after implantation of the morphine pellet. Brains, without the cerebellum, were homogenized in 10 volumes of 0.32 M sucrose using twenty strokes of a loosely fitting Teflon-glass homogenizer. The homogenate was centrifuged at 1,000g for 10 min and the supernatant fraction was centrifuged at 10,000g for 10 min. The pellet (P_2)¹⁴ was suspended in the original volume of 0.32 M sucrose and diluted with 8 volumes of 0.32 M sucrose, 0.01 M Tris-HCl, pH 7.4, for assay. Brains of three to six rats were pooled after homogenisation in each of four independent experiments, all of which gave equivalent results.

Narcotic drug binding was studied with the aid of ^3H -dihydromorphine, specific activity 51.2 Ci mmol^{-1} (New England Nuclear Corp.). Brain homogenates or, more usually, the 10,000g particulate fractions P_2 (0.5 mg of protein in 900 μl of 0.32 M sucrose, 0.01 M Tris-HCl, pH 7.4), were incubated with ^3H -dihydromorphine in the presence and absence of unlabelled morphine (or other drug) at a concentration such that the specifically bound dihydromorphine was displaced by the unlabelled narcotics. After 10 min at 37° C, unbound radioactive material was removed by centrifugation at 19,000 r.p.m. for 10 min. The tube and surface of the pellet was washed with 1 ml of 0.32 M sucrose and the washed pellet, suspended in 1 ml of 1% Triton X-100, was assayed for radioactivity. All operations involving dihydromorphine were carried out in the dark or in a room illuminated with extremely dim, indirect daylight. At 10^{-9} M dihydromorphine, as in Figs. 2–4, approximately half the bound radioactivity was specifically bound in that it was displaced by low concentrations of narcotics but not by their inactive stereoisomers.

Residual morphine present in the P_2 fraction of the homogenate of the brains of morphine-treated animals was estimated after extraction by the method of Mulé¹⁵. The organic solvent extracts, after removal of the solvent, were dissolved in a small volume of methanol. Aliquots were assayed for morphine by measuring the amount of bound ^3H -dihydromorphine displaced in our standard binding assay compared with standard morphine solutions. The total amount (free and bound) of residual morphine present in the P_2 fraction, 0.5–1.5 pmol per mg protein, was such that its concentration was less than 10^{-9} M when diluted as in our standard assay. Such concentrations could not affect our results in any important manner. Furthermore, repeated washings of the P_2 fractions did not change our results.

Figure 1 shows the specific binding of dihydromorphine to brain P_2 fractions prepared from normal and morphine-dependent rats. The two sets of data, identical within experimental error, show that both the number and binding affinity of the morphine receptor sites are the same in the control and morphine-dependent animals. Although the data are expressed per mg of protein they are also valid per rat, since the size and protein contents of the brains of the control and morphine-dependent rats were approximately the same.

Specificity of binding was assessed by measuring ^3H -dihydromorphine binding (at 10^{-9} M) at varying concentrations of narcotic analgesics (and other drugs). In all cases, the brain particulate fractions of normal and morphine-dependent animals were indistinguishable with respect to their binding properties. Thus, Fig. 2 presents data showing the competition of morphine for the receptor site and shows the identity of the two types of brain preparations. The narcotic antagonist naloxone also competes in an identical manner for the specific narcotic receptor sites of morphine-dependent and control rats (Fig. 3) as does the anticholinesterase, eserine (Fig. 4).

These experiments provide evidence that morphine dependence (and therefore also tolerance^{4,7}) is not the result of an alteration in the number or of the nature of the specific receptor sites in the brains of rats. Thus, this class of theories cannot account for narcotic drug dependency. In corroboration is the fact that dependent animals, tolerant to large doses of narcotic drugs, still react vigorously to small doses of antagonists such as naloxone or nalorphine^{7,12}. These are believed to bind to the

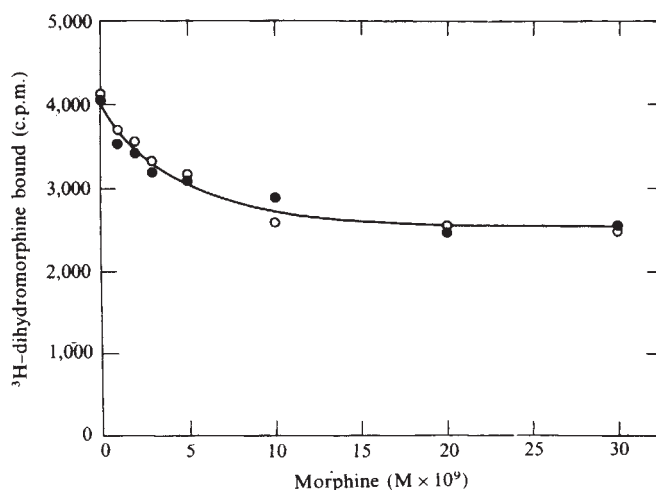


Fig. 2 The displacement of bound ^3H -dihydromorphine from particulate fractions of rat brain homogenates by morphine. Open circles, control; filled circles, morphine-dependent animals. Each flask in this and the subsequent figures contained 0.5 mg of P_2 protein, 9 μmol Tris-HCl, pH 7.4, 1 pmol ^3H -dihydromorphine (31,000 c.p.m.), and the indicated concentration of unlabelled drug in a total of 1 ml of 0.32 M sucrose. The points are single values, and the binding indicated is per flask.

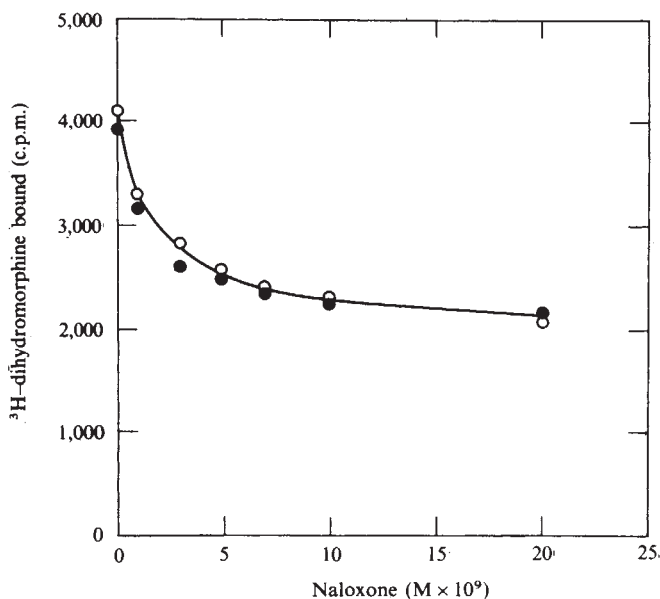


Fig. 3 The displacement of bound ^3H -dihydromorphine from particulate fractions of rat brain homogenates by naloxone. ○, Control; ●, morphine-dependent animals.

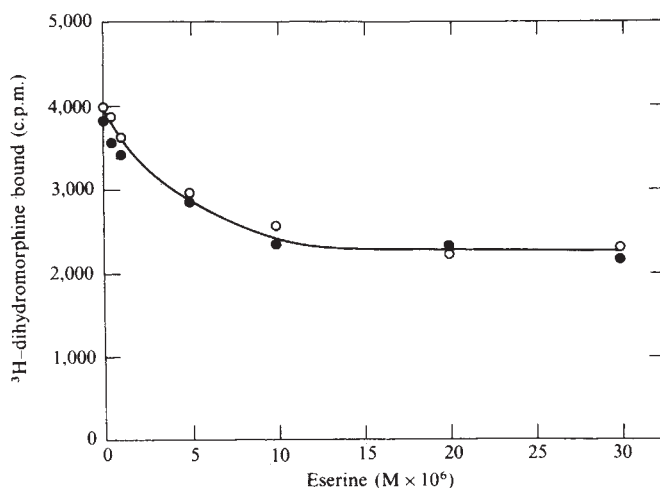


Fig. 4 The displacement of bound ^3H -dihydromorphine from particulate fractions of rat brain homogenates by eserine. ○, Control; ●, morphine-dependent animals.

same receptors as the narcotics themselves on the basis of *in vitro* binding studies⁸⁻¹⁰ and their very close structural similarity to the analgesics. Thus, if tolerance and dependence were simple the result of an increased number (or decreased affinity) of receptor sites much larger doses of antagonists should be required.

A body of evidence indicates that increased breakdown or neutralization of narcotics is also not responsible for tolerance. Thus, both brain and circulating levels of narcotic analgesics are generally similar or identical in normal and tolerant animals after administration of the same dose of a narcotic analgesic^{16,17}.

Apparently, narcotic drugs reach the brain and are bound at receptor sites to the same extent in both normal and tolerant animals. Tolerance and dependency therefore reflect a change in some subsequent process. Conceivably, the efficacy with which receptor binding is translated into the primary physiological response is greatly reduced in tolerant animals. Alternatively, physiological pathways which function in opposition

to the morphine-induced response may be greatly enhanced. This homeostatic mechanism is attractive in that it can easily predict the presence of strong withdrawal syndromes.

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Toxic action of a phalloidin-albumin conjugate on cells with a high protein uptake

PHALLOIDIN (PHD) is a toxic cyclopeptide in *Amanita phalloides*^{1,2}. Rats and mice injected with this toxin die in 2-4 h from hepatic necrosis. Dilatation of the endoplasmic reticulum (ER) with the appearance of very large vacuoles are the first ultrastructural changes in hepatocytes^{3,4}. The first biochemical lesion produced by PHD is unknown². Hepatocytes are the only cells damaged by PHD in mice and rats^{5,6}; Heart fibroblasts⁷ and bovine kidney cells⁸ cultured *in vitro* are not affected by this toxin.

Two possible explanations why PHD damages hepatocytes and not other cells are: (1) PHD enters only hepatocytes; (2) only within these cells does it find the target for its toxic action.

In previous experiments (ref. 8 and E. Bonetti, M. D. and L. F., unpublished) it was found that amanitin-albumin conjugates produce the characteristic changes of amanitin poisoning in cells which are not damaged by the free toxin^{8,9}, but which are very active in protein uptake such as the sinusoidal liver cells of mouse¹⁰ and the kidney proximal tubule cells of rat^{11,12}. This finding demonstrated that these cells are susceptible to amanitin but that the toxin is unable to penetrate them unless it is coupled to a protein and so forced to enter by pinocytosis.

We have conjugated PHD to bovine serum albumin (BSA) and studied the effects of the conjugate (PHD-BSA) on macrophages cultured *in vitro* and also on liver sinusoidal cells and kidney proximal tubule cells of mice. The con-

jugation of PHD with BSA was performed as described for the coupling of amanitin to BSA⁸. The molar ratio of phalloidin to albumin in PHD-BSA was calculated according to Wieland and Buku¹³ and was found to be 2.3.

The effects of PHD and of PHD-BSA on mouse peritoneal macrophages are reported in Table 1. It shows that conjugation with BSA produces a very strong increase in toxicity of PHD for these cells.

TABLE 1 Effect of PHD and PHD-BSA on mouse peritoneal macrophages.

PHD		PHD-BSA	
$\mu\text{g ml}^{-1}$ *	% dead cells	$\mu\text{g ml}^{-1}$ *	% dead cells
100	25	400 (12) [†]	100
50	10	200 (6)	100
25	0	100 (3)	75
12.5	0	50 (1.5)	75

Mouse peritoneal macrophages, obtained as previously described¹⁴, were incubated at 37°C in 0.5 ml of Eagle's basal medium (BME) containing PHD or PHD-BSA. After 15 h 2 ml of BME with 10% foetal bovine serum were added and after further 9 h of incubation the number of dead cells was determined by staining with Trypan blue¹⁵. In the PHD-BSA experiment most of the cells were detached from the glass at the end of the incubation period.

* Concentration during the first 15 h of incubation.

[†] In parentheses the amount of PHD contained in PHD-BSA.

To study the effects of PHD-BSA on liver sinusoidal cells and kidney proximal tubule cells, Swiss male mice were killed 8 or 24 h after intraperitoneal injection of PHD-BSA dissolved in 0.9% NaCl at a concentration of 800 $\mu\text{g ml}^{-1}$ and given at the non-lethal dose of 80 μg per 10 g body weight ($\approx 2.3 \mu\text{g PHD}$). Specimens of liver and kidneys were observed through the electron microscope.

Eight hours after injection of PHD-BSA, liver sinusoidal cells seemed damaged; the cisternae of rough ER were dilated, forming vesicles of different sizes. In some cells huge vacuoles surrounded by a single membrane occupied a large part of the cytoplasm (Fig. 1). They contained a fibrillar substance of low density, which was scattered in finely divided form. Many sinusoidal cells seemed detached from underlying hepatocytes whose microvilli jutted out directly into the sinusoidal lumen. No morphological alterations were found in nuclei. The hepatocytes seemed unchanged. Twenty-four hours after injection of PHD-BSA liver sinusoidal cells looked normal; they bordered the sinusoidal lumen everywhere.

In kidney PHD-BSA caused changes in the cells of the proximal convoluted tubules. The cells of every other portion of the renal tubular system were unaffected. This agrees with the finding that after injection of albumin in the mouse the only cells of renal tubular system which take up the protein are those of the proximal convoluted tubules¹¹. In these cells a marked increase in smooth ER was observed 8 h after PHD-BSA injection. After 24 h in many cells of the proximal convoluted tubules the cisternae of rough

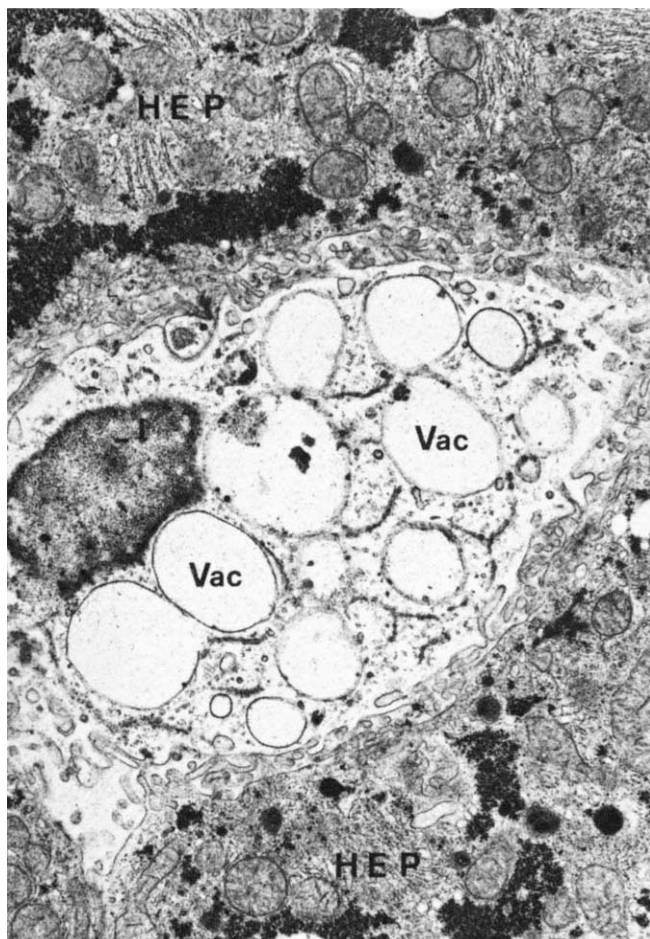


FIG. 1 Mouse killed 8 h after injection of PHD-BSA. Very large vacuoles (Vac) surrounded by a single membrane occupy large part of the cytoplasm of a liver sinusoidal cell. The cytoplasm of hepatocytes (Hep) looks normal. Fixation: 1% osmium tetroxide dissolved in 0.1 M Sorensen buffer, pH 7.2. Embedding: Araldite-Epon mixture according to Mollenhauer¹⁶. Staining: lead citrate. $\times 11,000$.

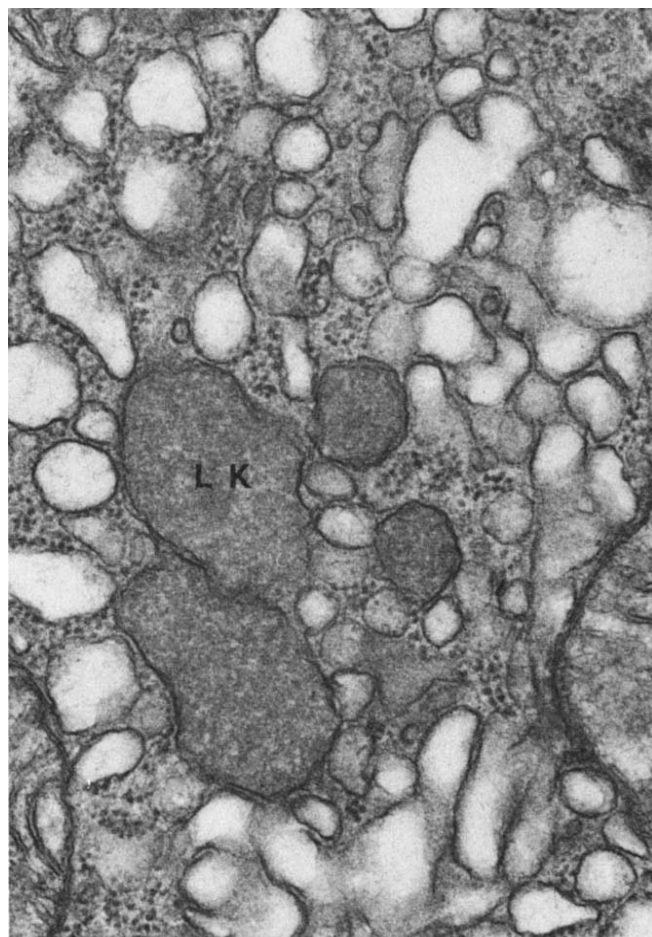


FIG. 2 Mouse killed 24 h after injection of PHD-BSA. Cytoplasm of a proximal tubule cell of kidney. The cisternae of rough ER form vesicles of different sizes. LK indicates Lipidkörperchen (see text). Fixation, embedding and staining as in Fig. 1. $\times 45,500$.

ER were dilated, forming vesicles of different sizes (Fig. 2). This lesion is very similar to that observed in hepatocytes 15–30 min after PHD administration^{3,4}. Bodies surrounded by a single membrane, about 0.2 μm in diameter containing moderately dense, homogeneous material, which are a frequent finding in hepatocytes of mouse poisoned with PHD (*Lipidkörperchen*)⁶ were always observed in cells with dilated rough ER (Fig. 2). Some cells instead of the vacuolisation of rough ER showed the increase of smooth ER which is a common finding 8 h after PHD-BSA administration.

Our results show that conjugation with BSA makes PHD toxic for cells with a high protein uptake. The lesions produced by PHD-BSA are very similar to those caused in hepatocytes by free PHD^{3,4}. Since proteins after penetration into cells are rapidly broken down by lysosomal enzymes¹⁷, it seems likely that PHD-BSA exerts its toxic action within the cells after digestion of the protein moiety and release of free PHD from the conjugate. These results indicate also that the absence of lesions in cells different from hepatocytes following PHD administration is due to the inability of these cells to take up the toxin. Therefore the finding that the cells of gastrointestinal mucosa of mice are unaffected after injection of large doses of PHD (20 mg kg⁻¹ i.p.) (our unpublished observations) suggests that the toxin cannot enter these cells and explains why mice are very resistant to the toxin when it is given orally².

These results as well as previous data on amanitin-albumin conjugates (ref. 8 and E. Bonetti, M. D., and L. F., submitted for publication) suggest a possible use of conjugates in biological research. Substances such as hormones and drugs have biological activity only in some cells and sometimes it is difficult to assess whether they enter only the cells in which they are active or whether only within these cells do they find the target of their action. For some of these substances this problem might be solved by conjugating them to a protein and by testing whether in cells with a high protein uptake, the conjugates produce the same effects as are caused by free substances in target cells.

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Sulphur-methylene isosterism in the development of metiamide, a new histamine H₂-receptor antagonist

BURIMAMIDE has been shown to antagonise the effects of histamine on isolated cardiac and uterine muscle *in vitro*, and to antagonise histamine-stimulated acid secretion *in vivo* in animals and in man¹. This pattern of pharmacological effects is not achieved by conventional, tertiary amine, anti-histaminic drugs (of which mepyramine is typical) and has led to the definition of burimamide as an H₂-receptor antagonist¹ and mepyramine as an H₁-receptor antagonist². Although burimamide was pharmacologically sufficiently active to allow for definition of its properties in man³ it seemed to lack the combination of high specific activity with adequate oral bioavailability needed for exploring the therapeutic potential of this new type of drug action. Attempts to produce a more suitable drug were based on the observation that burimamide is a competitive antagonist to histamine and that both compounds possess imidazole rings. We therefore compared the relative species populations of the respective rings and modified the burimamide structure so as to increase the equilibrium concentration of imidazole species considered most likely to be active.

In aqueous solution at physiological pH (7.4) burimamide exists as an equilibrium mixture of mainly three different imidazole species: the two uncharged tautomers A and B, and the cation C (Fig. 1, R = $-(\text{CH}_2)_4\text{NHCSNHCH}_3$).

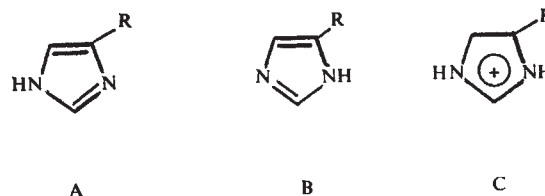
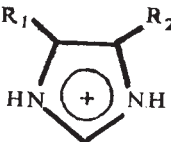


FIG. 1 Three species of imidazole with a side chain R at position 4(5).

Moreover this equilibrium probably involves a water-mediated proton transfer which is not instantaneous, for example protonation of imidazole at pH 7 has a pseudo-first-order rate constant of $1.5 \times 10^8 \text{ s}^{-1}$ (ref. 4). If only one of these forms were active its relative population could determine the amount of drug required for a given effect. The populations of these species are determined by the relative acidities (pK_a) of the two ionisable protons in the cation, but these are not directly measurable. They can be estimated, however, from the electronic influence of the side chain. The substituent R alters the electron densities at the ring nitrogen atoms and affects proton acidity⁵. Its effect is more marked at the nearer nitrogen atom so that if R is an electron releasing group, tautomer B should predominate; if it is an electron withdrawing group, A should predominate. The fraction present as cation C is determined by the ring pK_a and the pH of the solution⁶. The electronic influence of the side chain can be assessed from the measured ring pK_a using the Hammett equation⁷: $pK_{aR} = pK_{aH} + \rho\sigma_m$, that is, the pK_a of the ring substituted by R is reduced relative to imidazole (R = H) in direct proportion to the electron with-

TABLE 1 Apparent pK_a values of substituted imidazole cations at 37° C and their mol fractions (n_c) at pH 7.4

					
	R ₁	R ₂	pK_a	Preferred tautomer	n_c at pH 7.4
Histamine	H	$-\text{CH}_2\text{CH}_2\text{NH}_3^+$	5.90	A	0.03
Thiaborimamide	H	$-\text{CH}_2\text{SCH}_2\text{CH}_2\text{NHCSNHCH}_3$	6.25	A	0.07
Metiamide	CH_3	$-\text{CH}_2\text{SCH}_2\text{CH}_2\text{NHCSNHCH}_3$	6.80	A	0.20
Imidazole	H	$-\text{H}$	6.80		0.20
Burimamide	H	$-(\text{CH}_2)_4\text{NHCSNHCH}_3$	7.25	B	0.40
4(5)-Methylimidazole	H	$-\text{CH}_3$	7.40	B	0.50
Methylburimamide	CH_3	$-(\text{CH}_2)_4\text{NHCSNHCH}_3$	7.80		0.72

pK_a determined potentiometrically at 25° C on 0.005M solutions in 0.1 M KCl by titration against HCl, corrected to 37° C by subtracting 0.0225 units per ° rise for values in the range 7.5 to 7.0 (ref. 11), and 0.02 for the range 6.5 to 6.0 (ref. 12) and rounded off to the nearest 0.05 unit.

drawing effect (σ_m) of R (the reaction constant, ρ , being negative). The pK_a values of relevant compounds are in Table 1.

For histamine the ammonium-ethyl side chain ($R = -\text{CH}_2\text{CH}_2\text{NH}_3^+$) lowers the pK_a of the imidazole ring: it withdraws electrons and favours tautomer A. There is only a small proportion of cation C present at pH 7.4 (mol fraction $n_c = 0.03$); the main species is tautomer A, to the extent that approximately 80% of histamine molecules ($n_A = 0.80$) are in this form⁸. For burimamide the ring pK_a is greater than for imidazole, therefore the side chain ($R = -(\text{CH}_2)_4\text{NHCSNHCH}_3$) is electron releasing and tautomer B should be favoured. Electronically the side chain must resemble a methyl group since the pK_a is close to that of 4(5)-methylimidazole. At pH 7.4 the cation is one of the main species ($n_c = 0.40$) and tautomer A should be the least favoured.

Thus, although both histamine and burimamide are mono-substituted imidazoles, the structural similarity is misleading in that the predominant species of the respective imidazole rings are chemically different. If the active form of the antagonist were species A, the form most preferred for histamine, then activity might be enhanced by increasing its relative population. This could be done by converting the antagonist side chain into an electron withdrawing group, for example, by incorporating an additional electronegative atom in the side chain, preferably near to the ring. The disturbance to other molecular properties, such as stereochemistry and lipid-water partition, should however be kept minimal. One way would be to replace a methylene group ($-\text{CH}_2-$) by a thioether linkage ($-\text{S}-$). The data in Table 2 show that these are almost isosteric; they have similar van der Waals radii and give rise to similar bond

angles. The C-S bond is somewhat longer than a C-C bond so that in the thioether the adjacent methylene groups would be farther apart by 0.3 to 0.4 Å; this is quite a modest effect, amounting to an increase in distance between these two groups of about 15%. The thioether linkage may slightly increase conformational flexibility since the energy barrier to rotation of the C-S bond in the thioether linkage is lower than that of the C-C bond in the corresponding hydrocarbon. A sulphur atom is probably more hydrophilic than a methylene group; the octanol-water partition coefficient of diethyl sulphide ($\log P = 1.95$) suggests that the sulphur atom makes no additional contribution to the partition. Comparison with pentane ($\log P = 2.50$) shows that in this simple case replacement of CH_2 by S reduces partition by 0.5 log units, which amount is equivalent to the removal of a methyl or methylene group.

Making this substitution in burimamide at the carbon atom next but one to the ring gave the compound 'thiaborimamide' ($R = -\text{CH}_2\text{SCH}_2\text{CH}_2\text{NHCSNHCH}_3$). The effect on partition of the uncharged molecule is small. $\log P$ (at 37° C between 1-octanol and aqueous buffer at pH 9.0) is reduced from 0.39 (burimamide) to 0.16 (thiaborimamide). The electronic influence of the modified side chain, shown by the ring pK_a , is similar in magnitude but in the opposite direction to that of a methyl group. The main species should be tautomer A although its population may not be as large as it is in histamine because the electronic effect of the side chain is not as marked. A further increase in the population of tautomer A should be obtained by introducing an electron releasing substituent such as methyl in the vacant 4(5)-position of the ring, since electron releasing groups favour the form with the hydrogen atom on the adjacent nitrogen. The methyl group should not interfere with receptor interaction since it has been shown that 4-methylhistamine is an effective H_2 -receptor agonist¹. It might, however, exert an influence on the stereochemistry of the molecule by restricting rotation of the ring in a way analogous to that suggested to occur with 4-methylhistamine⁹. Incorporating methyl into the ring of the antagonist gave N-methyl-N'-{2[(5-methylimidazol-4-yl)methylthio]ethyl}thiourea, which has the name metiamide (name approved by the British Pharmacopoeia Commission and the US Adopted Names Council). The two ring substituents in metiamide are seen to have electronic effects of equal magnitude but of opposite direction. They should combine to favour tautomer A but oppose in their effect on ring pK_a ; indeed, they must exactly cancel since the pK_a s of metiamide and imidazole are identical. This means that at pH 7.4 the main species should be tautomer A, as for histamine, although there would still be a substantial proportion of cation present ($n_c = 0.20$). In comparison with burima-

TABLE 2 Comparison of methylene and thioether linkages

			
	X = CH ₂	X = S	Reference
C—X bond length (Å)	1.54	1.81	13, page 224
CXC bond angle, R = H	109°	105°	13, page 112
van der Waals radius of X (Å)	2.0	1.85	13, page 260
C...C interatomic distances			
between centres (Å)	2.51	2.87	
molar volume increment of X	16.58	10.78	14
C—X rotational barrier,			
R = H (calorie mol ⁻¹)*	3.3	2.13	15, 16
log P (octanol-H ₂ O), R = CH ₃	2.50	1.95	17, 18

* 1 calorie = 4.186 J

TABLE 3 H₂-Receptor antagonist activities. The dissociation constant (K_B) was calculated from the equation $K_B = B/(x - 1)$, where x is the respective ratio of concentrations of histamine needed to produce half-maximal responses in the presence and absence of different concentrations (B) of antagonist, and $-\log K_B = pA_2$.

	Atrium K_B (95% limits) $\times 10^{-6}$ M	Uterus K_B (95% limits) $\times 10^{-6}$ M
Metiamide	0.92 (0.74 - 1.15)	0.75 (0.40 - 1.36)
Thiaborimamide	3.2 (2.5 - 4.5)	3.2 (2.5 - 4.5)
Burimamide	7.8 (6.4 - 9.6)	6.6 (4.9 - 8.3)
Methylburimamide	8.9 (5.6 - 15)	10.7 (4.5 - 31)

mide, the ratio of tautomers is reversed for metiamide and the proportion of cation is decreased.

The pharmacological consequences of these manipulations are shown in Table 3. The effectiveness of each compound as an H₂-receptor histamine antagonist was compared by estimating the dissociation constant, K_B , (with 95% confidence limits) for the drug-receptor complex. The reliability of these estimates was tested by making separate measurements on two tissues, heart muscle and uterine muscle, which came from different animal species and which respond to histamine in different ways; heart muscle is stimulated and uterine muscle is inhibited. Further, the antagonism was shown to be specific by the failure to antagonise isoprenaline which apparently has identical pharmacological effects to histamine in these tissue¹. The results obtained on the two tissues are in good agreement and, taken together, show that metiamide *in vitro* is three to four times more active than thiaborimamide and eight to nine times more active than burimamide.

Thus it can be seen that modifying the side chain in burimamide by isosteric replacement of $-\text{CH}_2-$ by $-\text{S}-$ favours tautomer A, reduces the ring pK_a , and gives a more active compound (thiaborimamide). Introducing a 4(5)-methyl group to give metiamide increases further the preference for tautomer A but decreases the combined populations of the uncharged tautomers (A and B) through raising the ring pK_a . But although these are opposing effects the net result is that metiamide is more active still. By contrast, the analogous structural modification of burimamide, incorporation of a ring 4(5)-methyl group to give 'methylburimamide' does not increase activity. In this case the two ring substituents have nearly equal electronic effects in the same direction; the methyl group counterbalances the electronic influence of the side chain on tautomerism so that the two tautomers become equally populated, but it raises the ring pK_a to 7.80 so that at pH 7.4 the predominant species is the cation C ($n_c = 0.72$). This illustrates the problem of attempting to manipulate the biological properties of drug molecules through altering the structure. The changes in chemical properties accompanying structural modification often impose their own inherent limitations; a structural change, biologically advantageous with respect to a given chemical property, may affect some other chemical property in a biologically disadvantageous way and one has to discover the optimum balance of opposing influences. Finally, metiamide is not only a more active H₂-receptor antagonist than is burimamide *in vitro*, it is also more active *in vivo*. Metiamide has sufficient oral activity in animals and man as an inhibitor of evoked gastric acid secretion for consideration in therapy¹⁰.

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Similarity in the Sequence of *Escherichia coli* Dihydrofolate Reductase with Other Pyridine Nucleotide-requiring Enzymes

DIHYDROFOLATE reductase (DHFR) is a NADPH-requiring enzyme, and as isolated from a methotrexate-resistant strain of *E. coli* B^{1,2}, has two binding sites for NADPH³. The sequence of DHFR (Fig. 1) has been determined recently in our laboratory by standard methods including CNBr cleavage, enzymatic digestion, and manual Edman degradation with direct determination of the phenylthiohydantoins by gas chromatography and thin-layer chromatography. Novel methods used included free flow electrophoresis instead of ion exchange chromatography for the separation of peptides from tryptic digestion, and cellulose acetate electrophoresis to monitor separations of the peptides produced by CNBr. Details of this work will be reported elsewhere (C. D. B., J. A. Rodkey and J. M. Sondey, manuscript in preparation).

Engel⁴ has pointed out the similarities between the sequence of amino acids around the lysine active in cofactor-binding of glutamate dehydrogenase (GDH) with a second sequence in GDH, which he suggested might be the site of 'regulator'

```
Met- Ile- Ser- Leu- Ile- Ala- Ala- Leu- Ala- Val-
Asp- Arg- Val- Ile- Gly- Met- Glu- Asn- Ala- Met-
Pro- Trp- Asn- Leu- Pro- Ala- Asp- Leu- Ala- Trp-
Phe- Lys- Arg- Asn- Thr- Leu- Lys- Asp- Pro- Val-
Ile- Met- Gly- Arg- His- Thr- Trp- Glu- Ser- Ile-
Gly- Arg- Pro- Leu- Pro- Gly- Ser- Lys- Asn- Ile-
Ile- Leu- Ser- Ser- Gln- Pro- Gly- Thr- Asp- Asp-
Arg- Arg- Val- Thr- Trp- Val- Lys- Asn- Val- Asp-
Glu- Ala- Ile- Ala- Ala- Cys- Gly- Gln- Val- Pro-
Met- Val- Ile- Gly- Gly- Arg- Val- Tyr- Glu-
Gln- Phe- Leu- Pro- Lys- Ala- Gln- Lys- Leu- Tyr-
Leu- Thr- His- Ile- Asp- Ala- Glu- Val- Asp- Gly-
Asp- Thr- His- Phe- Pro- Asn- Glu- Tyr- Pro- Glu-
Trp- Glu- Ser- Val- Phe- Ser- Glu- Phe- His- Asn-
Ala- Asp- Ala- Gln- Asn- Ser- His- Tyr- Cys- Phe-
Lys- Ile- Leu- Glu- Arg- Arg-
```

Fig. 1 Sequence of dihydrofolate reductase of a mutant of *E. coli*.

G3PDH	198	Gly	Ala	Ala	Gln	Asn	Ile	Ile	Pro	Ala	Ser	Thr	Gly	Ala	Ala	Lys	Ala	Val	Gly	Lys	Val	Ile	Pro	221
	204																							227
ADH		Gly	Leu	Ser	Val	Ile	Met	Gly	Cys	Lys	Ala	Ala	Gly	Ala	Ala	Arg	Ile	Ile	Gly	Val	Asp	Ile	Asn	Lys
	267																							290
GDH-R		Gly	Ala	Lys	Cys	Val	Ala	Val	Gly	Glu	Ser	Asp	Gly	Ser	Ile	Trp	Asn	Pro-	Asp	Gly	Ile	Asp	Pro	Lys
	112															*								135
GDH-A		Thr	Tyr	Lys	Cys	Ala	Val	Val	Asp	Val	Pro	Phe	Gly	Gly	Ala	Lys	Ala	Gly	Val	Lys	Ile	Asn	Pro	Lys
	83																							106
DHFR		Ile	Ala	Ala	Cys	Gly	Gln	Val	Pro	Met	Val	Ile	Gly	Gly	Gly	Arg	Val	Tyr	Glu	Gln	Phe	Leu	Pro	Lys
	87															*								111
LDH		Val	Ser	Ala	Gly	Ser	Lys	Leu	Val	Val	Ile	Thr	Ala	Gly	Ala	Arg	Gln	Gln	Glu	Gly	Glu	Ser	Arg	Leu

* Residues known to be active in cofactor binding. The residue position in the whole protein is given above each sequence.

Fig. 2 Comparison of partial sequences of pig glyceraldehyde-3-phosphate dehydrogenase (G3PDH), horse alcohol dehydrogenase (ADH), the area of beef glutamate dehydrogenase suggested to be the regulator sequence (GDH-R), the same enzyme containing the lysine active in cofactor binding at 126 (GDH-A), *E. coli* dihydrofolate reductase (DHFR), and dogfish lactate dehydrogenase (LDH). Homologies with DHFR are shown in boxes.

activity by NADH. He also noted a similar sequence in glyceraldehyde-3-phosphate dehydrogenase (G3PDH). I wish to call attention to residues 83 to 106 of DHFR which resemble a portion of those sequences, and also to point out similar sequences in lactate dehydrogenase (LDH) and in alcohol dehydrogenase (ADH), two other enzymes which require pyridine nucleotides as cofactors.

Major portions of the sequences of only four other enzymes requiring pyridine nucleotides have been published. They are: pig glyceraldehyde-3-phosphate dehydrogenase (G3PDH)⁵; horse alcohol dehydrogenase (ADH)⁶; bovine glutamate dehydrogenase (GDH)⁷ with the lysine active in cofactor binding in position 126⁸; and dogfish shark M₄ lactate dehydrogenase (LDH)⁹ with the arginines active in cofactor binding in positions 101 and 109¹⁰. Sequences of G3PDH are also known from chicken, lobster and yeast. The difference between these three sequences and G3PDH from pig are minor and do not affect the homologies discussed below.

Portions of the relevant sequences are aligned in Fig. 2. The first two enzymes are aligned as suggested by Engel⁴. LDH is placed with one of the cofactor-binding arginines aligned with the cofactor-binding lysine of GDH, while DHFR and ADH were aligned by inspection.

Although it is hazardous to compare three dimensional structures based on one dimensional sequences, the similarities of the six sequences as aligned in Fig. 2 are striking. DHFR has 11 residues which appear in corresponding positions in at least one of the other enzymes, and of these residues 9 appear in the corresponding positions in two or more of the other enzymes. Also noteworthy are the three consecutive residues of either Gly or Ala in all sequences (except the 'regulator' site of GDH) just preceding the active site Lys or Arg and the charged residue seven or eight residues from the active site Lys or Arg.

There are a total of 63 identities among the six sequences. Statistical analysis (performed by Dr N. I. Bohidar) showed that 30 sets of six sequences randomly generated from the amino acid compositions of each of the sequences in Fig. 2 contained an average of 29.4 identities with a standard error of 1.203 (N=30). The 99.95% lower and upper confidence limits (24.9, 33.8) do not include the value of 63, which is the number of identities associated with the sequences in Fig. 2. The probability of the identities in Fig. 2 occurring by chance is less than 0.001.

These five enzymes have different substrates and are derived from different organisms. It is attractive to speculate that the common factor which explains the similarities which we have found is their common requirement for a pyridine nucleotide cofactor.

Current studies of active site labelling, additional sequences of enzymes requiring a pyridine nucleotide as a cofactor and

structure determination by X-ray diffraction should serve to test this proposal.

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Reduction of Denervation Supersensitivity of Muscle by Submechanical Threshold Stimulation

IN normally innervated adult muscle, membrane sensitivity to acetylcholine (ACh) is localised to the endplate and tendon regions^{1,2}. After surgical denervation, this sensitivity spreads over the entire membrane surface reaching a peak within 5 to 7 d (ref. 3). Two hypotheses have been proposed to explain the restriction of ACh sensitivity in normally innervated muscle. The first suggests that a trophic substance is released from the motoneurone, restricting transmitter sensitivity to the endplate region^{4,5}. The second hypothesis suggests that muscle 'activity' generates a self-feedback loop from the muscle onto the sarcolemma thus limiting ACh sensitivity to the neuromuscular junction^{3,6}. Upon denervation, muscle 'activity' ceases and therefore the feedback loop is broken with the consequent loss of activity-mediated transmitter sensitivity restriction. Drachman and Witzke⁷ showed that direct electrical

stimulation of the denervated rat diaphragm, resulting in contraction, suppressed the spread of ACh supersensitivity. Jones and Vrbová⁶ reported that chronic electrical stimulation of the extensor digitorum of rat, resulting in contractions, reduced supersensitivity to ACh subsequent to denervation.

If muscle 'activity' is responsible for limiting ACh sensitivity to the endplate region, then feedback information from the muscle could emanate either from the contractile event or from membrane events in the absence of excitation-contraction coupling. To determine if mechanical activity, *per se*, is a necessary condition for limiting ACh sensitivity, or whether fluctuations in membrane potential alone constitute a sufficient signal for transmitter sensitivity restriction, we have stimulated denervated muscle above and below the mechanical threshold.

Both extensor digitorum longus (EDL) muscles from albino rats weighing 300 to 400 g were denervated by removal of a 1 to 2 cm section of the deep peroneal nerve between the EDL and the knee⁵. Teflon coated, multistrand wire (32 gauge) was wrapped once around the tendon of origin and insertion and secured by a nylon ligature to nearby connective tissue. The wire was fed through a small diameter polyethylene tube, previously introduced subcutaneously from the thigh to the neck, where it was brought to the surface for connection to a stimulator. One EDL of the first group of animals was electrically stimulated with 5 ms pulses, at a frequency of 15 pulses s⁻¹ and an amplitude of 6.6 ± 0.1 V for 1 h d⁻¹ over a period of 4 d commencing 24 h after denervation. Stimulus intensity was adjusted to a level resulting in maximal contractions as observed with a surgical microscope at × 80 magnification. The other

1 × 10⁻⁵ M ACh. After a washout period of 5 min in RR, the muscles were challenged with a solution containing 1 × 10⁻³ M ACh. Isometric tension was measured with two RCA 5734 mechano-electric transducers and recorded on a Hewlett-Packard paper recorder. Tension measurements were standardised by calculating tension per unit wet muscle weight. The results from paired muscles are shown in Table 1. The data show that sensitivity to ACh from subliminally stimulated muscles is consistently decreased by up to 38% when compared to sham stimulated muscles. The data also indicate that there is no difference between subliminally stimulated and maximally activated muscles.

On this basis we conclude that the feedback information from the muscle onto its sarcolemma is not necessarily mediated by activation of the contractile machinery itself. Muscle membrane potential fluctuations, induced by subliminal electrical stimulation, are therefore sufficient to restrict the spread of denervation-induced transmitter supersensitivity. The reduction of supersensitivity by subliminal stimulation must represent a minimum value as it is quite likely that centrally located fibres did not receive sufficient current to be affected. Therefore, if we were to eliminate the contribution to ACh sensitivity of these centrally located fibres, the reduction in sensitivity would be even greater than we report here. This consideration, no doubt, also contributes to the rather large variability in the response of different muscles owing primarily to the different current densities which depend on muscle geometry. Our results confirm earlier reports^{3,7} that electrical stimulation of denervated muscle is effective in limiting the spread of denervation-

Table 1 ACh mediated isometric tension of paired denervated muscles

Test solution *	Sham †	Against	Subliminal	% Difference	Maximal	Against	Subliminal	% Difference
1 × 10 ⁻⁵ M ACh	3.1 ± 0.5 ‡		1.9 ± 0.4	-38§	1.4 ± 0.4		1.4 ± 0.6	0
1 × 10 ⁻³ M ACh, first challenge	33.1 ± 7.2	n=5	26.6 ± 6.3	-20	11.4 ± 1.5	n=11	11.3 ± 1.9	0
1 × 10 ⁻³ M ACh, second challenge	12.6 ± 3.3	n=5	8.4 ± 4.3	-33	4.0 ± 1.4	n=11	3.8 ± 1.0	0

* Acetylcholine was dissolved in rat Ringer solution having the following composition: 160 mM Na⁺, 5 mM K⁺, 2 mM Ca²⁺, 1 mM Mg²⁺, 146 mM Cl⁻, 15 mM HCO₃⁻, 5 mM Tris buffer, 5 mM HPO₄²⁻, and 11 mM glucose at pH 7.1, saturated with 95%-5% O₂-CO₂.

† All muscles were denervated and connected to the stimulator wires in an identical manner. No stimulating current was delivered to the sham muscles. Stimulated muscles received trains of rectangular pulses lasting 5 s. Pulses within the trains had a duration of 5 ms and a frequency of 15 s⁻¹. The amplitude of the pulses delivered to the subliminal muscles was adjusted to a level just below any visually detectable mechanical activity, whereas maximal muscles received stimuli whose amplitude resulted in maximal mechanical activation. n=number of muscle pairs.

‡ Values given are means ± S.E.M. in g tension per g wet muscle weight.

§ Significant at the 0.05 level.

EDL of each animal was stimulated in the same manner except that stimulus intensity was adjusted to just below the mechanical threshold (3.9 ± 0.1 V) as judged from frequent microscopic observations of superficial fibres.

One muscle from a second group of animals was stimulated for 48 h continuously with trains of square pulses lasting 5 s. Pulses within the train had a duration of 5 ms, a frequency of 15 s⁻¹ and a pulse amplitude of 2.9 ± 0.3 V. This amplitude was just below the mechanical threshold (subliminal stimulation). The inter-train interval was 7 s. The other muscle of each animal served as a control. It was denervated and connected to the stimulating wire as described above except that no current was passed to that muscle (sham stimulation). Two hours after the end of the stimulation regime, both muscles from the same animal were excised and mounted in a constant temperature chamber at 37 ± 1° C. The muscles were continuously superfused with rat Ringer (RR) solution saturated with 95% O₂, and 5% CO₂ at a flow rate of 40 ml min⁻¹ (Table 1). The muscles were stretched to a resting tension of 5 g and equilibrated in RR for 5 min before being challenged with RR solution containing

induced supersensitivity. In addition, our data favour the hypothesis that sarcolemmal sensitivity to ACh is regulated by a mechanism located in or near the muscle membrane. This mechanism could be activated by fluctuations in membrane potential without the necessary involvement of excitation-contraction coupling.

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Role of Muscle Activity in Nerve-Muscle Interaction *in vitro*

CLONAL nerve and muscle cells interact *in vitro* to produce a nerve-muscle contact which is associated with an area of increased acetylcholine sensitivity on the muscle^{1,2}. This association is reminiscent of the distribution of acetylcholine sensitivity on innervated skeletal muscle *in vivo*^{3,4}. It is possible to use cultures of nerve and muscle cells to examine the requirements for localization *in vitro*. A previous report showed that cholinergic transmission is not required². Here I discuss the requirement for muscle electrical and contractile activity in localisation of acetylcholine sensitivity.

To examine the requirement for muscle activity in the events leading to localisation, it was necessary to block the electrical and contractile activity in muscle fibres. The action potential is resistant to 3×10^{-7} M tetrodotoxin⁵ and 5 mM procaine was toxic on prolonged exposure. However, both the action potential and delayed rectification are inactivated by prolonged depolarisation (Y. Kidokoro, in preparation), as is the case in frog skeletal muscle fibres⁶. The resting membrane potential is largely determined by a K^+ permeability, so it is possible to depolarise the cells by increasing the external K^+ concentration. When the external K^+ was increased from 5.3 to 25.3 mM by adding sterile 1 M KCl to growth medium⁷, the membrane potential was depolarised to about -40 mV and remained depolarised up to 2 weeks (-39 ± 5 mV, $N=48$). The resting potential in 5.3 mM K^+ is about -70 mV.

Muscle fibres remained depolarised throughout a long term exposure to 25.3 mM K^+ medium, but it was necessary to show that action potentials were still blocked. Accordingly, muscle fibres grown in medium with 25.3 mM K^+ (high K^+) for 10–14 d, were penetrated with two microelectrodes while still in high K^+ . To elicit even a partial action potential, a fibre had to be polarised to at least -70 mV for 100 or more ms

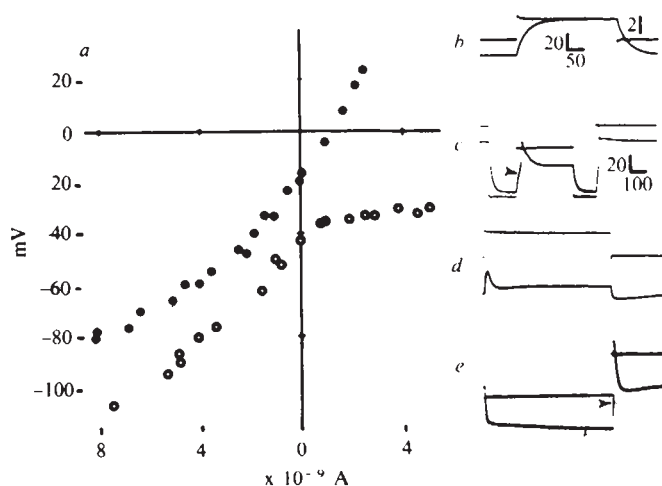


Fig. 1 The steady-state current-voltage relationship (measured 200 ms after the start of the current pulse) for a muscle exposed to high K^+ medium for 12 d and studied in high K^+ , is shown in (a) (solid circles); that for a muscle fibre fused in high K^+ , grown for 14 d, then studied in normal K^+ is shown by hollow circles. Samples traces from the same fibres are shown. (b) Shows the absence of delayed rectification in high K^+ , while (d) shows its presence in the fibre formed and grown in high K^+ . (c) Shows that only a partial action potential can be evoked from this fibre in high K^+ (a hint of the potential can be seen at the end of the hyperpolarizing pulse, but it is more clearly shown by the superimposed depolarization). (e) Shows an overshooting action potential in the fibre fused in high K^+ , then studied in normal K^+ . Note that the inflections in the rising phase of the action potentials occur at -50 to -60 mV (arrows). Calibrations are shown in the figure—the upper trace shows injected current ($\times 10^{-9}$ A) and zero membrane potential, the lower shows membrane potential (mV), time is in ms. The calibrations are the same for (c), (d) and (e).

(Fig. 1c). The steady state current-voltage relationship in high K^+ showed anomalous rectification, rather than delayed rectification (Fig. 1a). No permanent changes had been made in membrane properties, however, since when fibres exposed to high K^+ for 2 weeks were washed and studied in normal K^+ medium, they immediately (that is, the first cell penetrated) could generate overshooting action potentials on anode break stimulation, and showed delayed rectification. Furthermore, myoblasts grew and fused normally in high K^+ . Action potentials and delayed rectification were inactivated in high K^+ in these fibres, as just described for fused fibres chronically exposed to high K^+ . Again, if such fibres (fused in high K^+) were washed and studied in normal K^+ , they also could generate overshooting action potentials (Fig. 1e) and showed delayed rectification (Fig. 1a and d).

Table 1 Average Enhancement of Sensitivity at Nerve-Muscle Contacts

No. of muscle fibres	Ratio of peak localised sensitivity to background (Average)	(Range)	No. of sites tested per cell (Average)	(Range)	Success, 0.7
11	18	6–44	23	8–63	0.7

L6 myoblasts⁸ and N18 neuroblastoma cells⁹ were cultured as described previously². The distribution of acetylcholine sensitivity was determined by penetrating a muscle fibre with a 3 M KCl-filled micropipette (70–150 M Ω), then applying acetylcholine iontophoretically from an extracellular pipette filled with 2.7 M acetylcholine chloride (100–300 M Ω)². The acetylcholine sensitivity of the membrane is defined⁴ as the membrane depolarisation (in mV) divided by the amount of charge (in nC) passed through the acetylcholine pipette to produce the response (mV nC⁻¹). The results are pooled for fibres which had the 25.3 mM K^+ medium added before or after myoblast fusion, since localisation occurred in either case. The number of sites tested per cell gives the number of different areas of membrane that were tested with applied acetylcholine. 'Success' is the ratio of the number of muscle fibres that showed localisation to the number of nerve-muscle pairs that were chosen for study on the basis of their appearance in the phase microscope. The site of contact had to be clearly visible and situated so that the acetylcholine sensitivity could be determined all around it, and it had to be possible to trace the nerve process back to a nerve cell body.)

High K^+ medium reversibly stopped the twitching of spontaneously active muscle fibres. Fibres in high K^+ did not contract in response to intracellular stimulation or iontophoretically applied acetylcholine. These observations are consistent with studies on frog muscle fibres, which first contract when exposed to an increased K^+ concentration, then relax and no longer respond to electrical stimulation¹⁰.

To determine whether muscle activity was required in the events leading to localisation of acetylcholine sensitivity *in vitro*, nerve and muscle cells were cultured together in high K^+ medium for 8–12 d. In some studies the high K^+ was added to cultures of myoblasts before they had fused. In all studies high K^+ medium was added before the nerve cells and all electrophysiology was done in high K^+ medium, so that the block of activity was in effect throughout the experiment. Many examples of localisation of acetylcholine sensitivity were found, as seen in Fig. 2 and Table 1. In each case the peak of sensitivity was found at a nerve-muscle contact, and there was a clear gradient of sensitivity across the muscle surface up to the peak (see ref. 2 for further discussion).

It concluded from these results that muscle action potentials and contractile activity are not required for the events leading to the association of a nerve-muscle contact with an area of increased acetylcholine sensitivity on the surface of the muscle in this clonal system. Muscle fibres in primary cultures of chick embryo tissue can generate areas of increased acetylcholine sensitivity¹¹, or receptor density¹² in the absence of nerve cells. It is not known whether the localised sensitivity seen at a point of nerve-muscle contact in clonal cell culture preceded the contact or was induced by the intercellular contact.

The results obtained when high K^+ medium was added to myoblasts before fusion show that, in either case, muscle activity was not required for its production.

Recent studies have shown that electrical stimulation of denervated muscle *in vivo*^{13,14} can reduce the extrajunctional sensitivity, and stimulated muscle fibres *in vitro* are less sensitive than tetrodotoxin-treated fibres¹⁵. These studies strongly suggest that muscle activity influences the extrajunctional sensitivity on muscle fibres. However, results which are difficult to reconcile with the conclusion that muscle activity strictly determines the extrajunctional sensitivity have been reported¹⁶⁻¹⁸, and the relationship between muscle activity and sensitivity is not clear at present. Specifically, although activity reduced the extrajunctional sensitivity of denervated muscle, no effect on junctional sensitivity was noted¹³. It is possible that junctional and extrajunctional sensitivities are controlled by different processes and might be differently affected by muscle activity. It is not known whether the localisation seen in this clonal cell culture involves a decrease in the background sensitivity on the muscle cell (further discussion in ref. 2). Thus, there is no contradiction between the present results and other studies¹³⁻¹⁵.

My results demonstrate that muscle activity is not required for the localisation of acetylcholine sensitivity on these fibres *in vitro*, but do not indicate whether muscle activity has other effects on sensitivity (perhaps on the extrajunctional sensitivity, for example, refs 13-15).

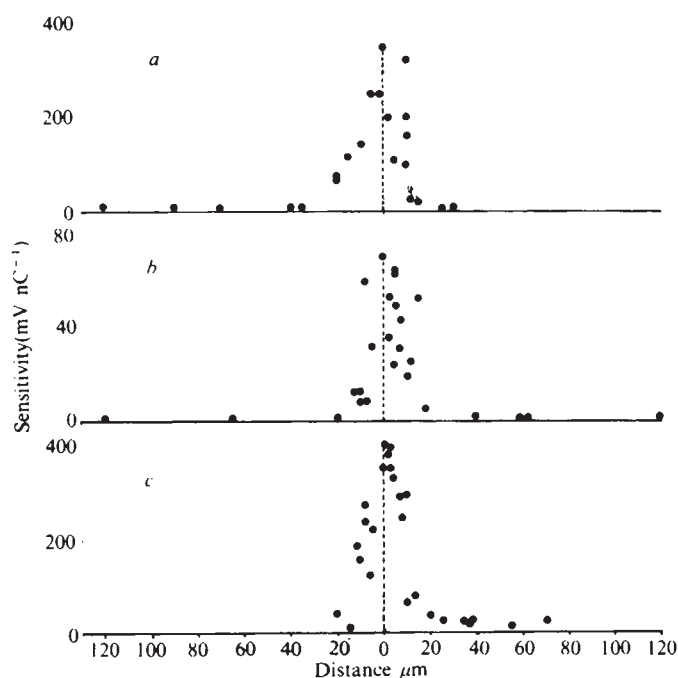


Fig. 2 Examples of localization of acetylcholine sensitivity. The ordinate shows the acetylcholine sensitivity (in $mV nC^{-1}$) detected at various points on the muscle surface, while the abscissa shows the distance in μm between the indicated site and the site with the maximal sensitivity. In each case, the peak of sensitivity was at a nerve-muscle contact. In (a) and (b), the 25.3 mM K^+ solution had been added to cultures of fused muscle fibres; in (c) the 25.3 mM K^+ solution had been added to a culture of myoblasts before fusion.

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Spine Loss and Regrowth in Hippocampus following Deafferentation

SEVERAL studies have reported that there is a loss of dendritic spines after partial deafferentation. It is known that terminals lost to a structure after injury to one of its afferents are replaced in part or whole by sprouting of undamaged inputs¹⁻³. This raises questions as to the nature of the accompanying post-synaptic changes but no attempts have been made to follow the loss of spines over time⁴⁻⁶. Specifically, there are no data on whether spines are replaced when sprouting afferents invade deafferented dendritic zones. This is a critical question if the nature of the morphological reorganisation which follows lesions in the brain is to be understood.

We therefore studied the effects of lesions of the entorhinal cortex on the spine population of neurones in the dentate gyrus. The entorhinal cortex generates the temporo-ammonic tract⁷⁻¹⁰, which contributes more than half of the synapses in the outer molecular layer of the dentate gyrus (D. A. Matthews, C. W. C., and G. L., unpublished). Following its removal, axons of the commissural system (which occupies the inner molecular layer) and the crossed temporo-ammonic tract (which terminates on the apical dendrites of CA1 pyramids) invade the outer molecular layer and form functional terminals¹¹⁻¹⁴. The scattered AChE terminals, probably originating in the septum and located in the outer molecular layer, proliferate after entorhinal lesions¹⁵⁻¹⁶. Thus, considerable replacement of the temporo-ammonic tract terminals occurs in the outer molecular layer after entorhinal lesions. Here we report changes which occur on dendritic spines of cells in the molecular layer while this axonal growth takes place.

Large electrolytic lesions were placed in the entorhinal cortex of adult rats of both sexes. The animals (E) were sacrificed at 5 ($n=4$), 20 ($n=4$), 60 ($n=4$) and 100 ($n=4$) d. An equal number of animals of the same age and approximately of the same weight served as controls (C). The rats were anaesthetised with ether. Tissue was excised from the rostral hippocampus and immediately immersed in rapid Golgi fixative of osmium tetroxide and potassium dichromate for 6 d. After fixation, the blocks were placed in 0.75% silver nitrate for 24 h. They were then 'shelled' in paraffin and cut on a sliding microtome at 75 μm . Following two washes each with absolute ethanol, methyl salicylate and xylene, they were mounted under Per-

mount. The rapid Golgi method silhouettes the soma-dendritic complex in detail including the dendritic spines¹⁷.

Spines were counted in the outer and inner molecular layers of the dorsal leaf of the dentate gyrus ipsilateral to the entorhinal lesions. Counts were performed on 45 μm segments (stations) on fifteen granule cells per animal (Fig. 1). All measurements in the outer molecular layer were taken from dendritic branches located within 100 μm of the hippocampal fissure and those for the inner molecular layer were made within 100 μm of the granule cell layer. As far as possible the same medial-lateral segment of dentate gyrus was examined in each rat.

Spine density was essentially the same in both layers in normal rats (Table 1). Five days after the entorhinal lesion there was a 30% reduction in the number of spines of the outer molecular layer (Fig. 2) but no changes were found in the inner molecular layer. By 20 d after the lesion, spine density had returned to 80% of normal and was back to control levels 60 d after the lesion (Table 1).

Table 1 No. of Dendritic Spines per μm of Dendrite

Station	Controls (C)	Experimental (E)	% difference *	P †
5 d after lesion				
A	0.64 ± 0.12 ‡	0.60 ± 0.09	-4.7	—
B	0.61 ± 0.10	0.43 ± 0.10	-29.5	<0.005
20 d after lesion				
A	0.60 ± 0.10	0.63 ± 0.10	+3.3	—
B	0.60 ± 0.11	0.48 ± 0.11	-20.0	<0.005
60 d after lesion				
A	0.62 ± 0.12	0.60 ± 0.09	-3.2	—
B	0.60 ± 0.09	0.60 ± 0.11	0.0	—
100 d after lesion				
A	0.63 ± 0.10	0.64 ± 0.11	+1.6	—
B	0.61 ± 0.11	0.60 ± 0.10	-1.6	—

* $100 (E \text{ mean} - C \text{ mean}) / C \text{ mean}$.

† Student's *t* test.

‡ Standard deviation.

It is interesting to compare the time course reported above with that reported for the invasion of axonal sprouts into partially denervated dendritic territory. Raisman and Field reported that the terminal population of dendritic spines in the

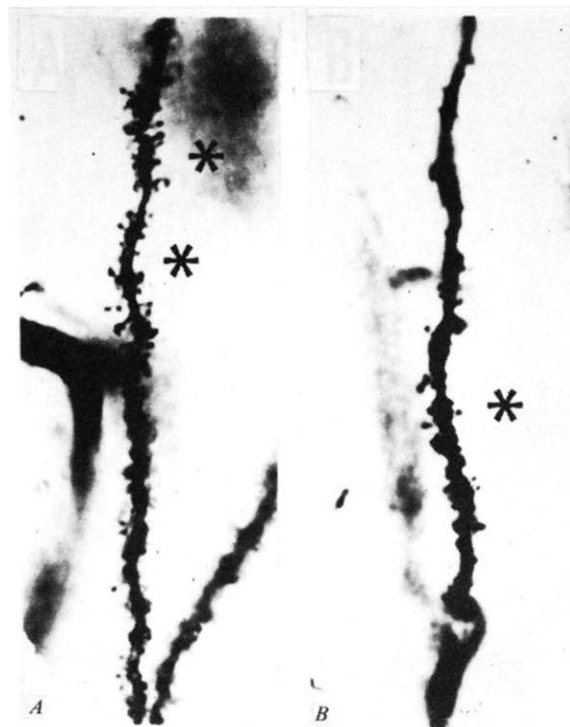


Fig. 2 A, Density of spines in the outer molecular layer of a normal adult rat; B, loss of spines as a result of an electrolytic lesion in the entorhinal cortex 5 d after the operation. * Portions of dendrite in focus.

septal nuclei was reduced to one-half of normal values 7 d after section of their hippocampal projections but returned to normal values by 30 d after the lesion¹⁸. The new terminals are thought to arise in part from sprouting of medial forebrain bundle axons. Neurophysiological studies have shown that the crossed temporo-ammonic¹⁴ and commissural (J. West, S. Deadwyler, C. W. C., and G. L., unpublished) systems establish functional synapses in the outer molecular layer 10 to 14 d after a lesion to the entorhinal cortex. Finally, histochemical experiments indicate that the process of axon sprouting is begun 5 to 7 d after lesions¹⁹. Taken together, these findings indicate that axon sprouting starts within 7 d of a lesion, establishes some functional synaptic contacts by 14 d after the lesion, and is essentially completed within 30 d of the lesion.

Although our data do not provide a precise picture of the time course for spine regrowth it is evident that the process is occurring during the period in which axonal sprouting takes place. This leads to the suggestion that the two processes, namely spine regrowth and axonal sprouting, might be related. Spine formation during development is often hypothesized to be dependent upon the arrival and normal function of axon terminals. We suggest that a similar mechanism occurs in the adult. A reduction of afferents results in loss of spines. Replacement of afferents by sprouting generates a new spine population. A special case of this is data reported by Valverde²⁰ from a study in which he raised mice in complete darkness from birth to 20 d and then placed them under normal lighting conditions for periods of up to 30 d. He claims 'recovery' of some spines on apical dendrites in the visual cortex of these animals—and suggests that the development of this group of spines results from the arrival of normal visual inputs. The possibility exists that there may be little or no correspondence between the two studies.

Our hypothesis, stated above, might also explain why several workers have observed a permanent reduction in spine densities following lesions^{5,6}. There is evidence that post-lesion sprouting is not a universal effect^{21,22}. If some brain regions are not re-

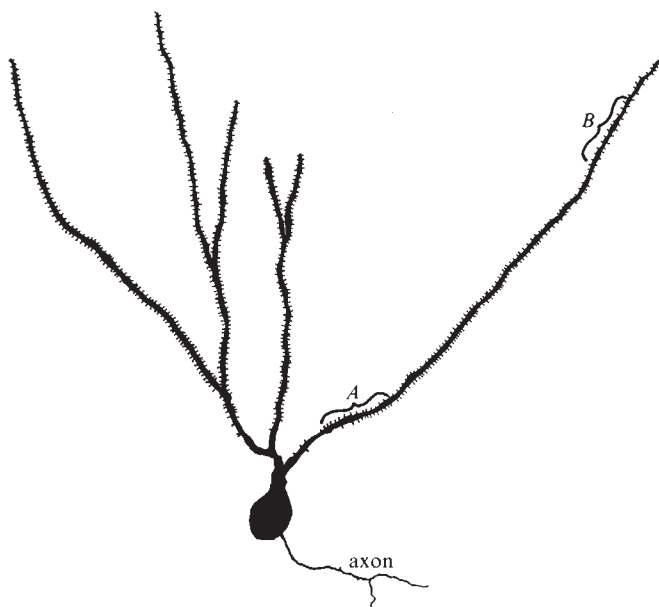


Fig. 1 Counting stations along the dendrites of the hippocampal granule cell. Stations A and B are located in the inner and outer molecular layers of the dentate gyrus respectively.

innervated by sprouting after the removal of afferents, then spine loss should be permanent.

Finally, these experiments provide clues as to factors which regulate spine density. The dendritic zones deafferented by the entorhinal lesions are re-innervated by three and possibly more different axonal systems. If spine density were solely dependent on the growth potential of presynaptic elements it would hardly be expected that these diverse inputs would generate the same number of spines as the single afferent they replace. Yet our data indicate that spine densities return to values essentially identical to pre-lesion conditions. A more parsimonious hypothesis is that granule cells in the hippocampus are capable of producing a certain number of spines. Given that an adequate number of terminals is present, these cells will generate this quantity of spines independent of the specific type of afferents.

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Growth Hormone Releasing Factor of Microbial Origin

CHOLERA enterotoxin mediates specific biochemical events in both intestinal and non-intestinal tissues by stimulating adenyl cyclase and cyclic AMP^{1,2}. Other evidence points to a role for cyclic AMP in regulating the secretion of several hormones by their respective endocrine glands³. These findings have led us to investigate whether cholera enterotoxin affects the secretory function of pituitary cells.

Cholera enterotoxin, an extracellular protein of molecular weight 84,000, was purified from culture filtrates of *Vibrio cholerae*, Inaba 569B, as described earlier⁴, adding Sephadex-G-150 chromatography as a final step. Absence of residual somatic antigen was established by the failure of detoxified enterotoxin preparations to elevate vibriocidal antibody; and absence of choleraenoid, a degradation product of toxin⁵, was shown by acrylamide gel electrophoresis.

Monolayer cultures of enzymatically dispersed cells from whole pituitaries of Charles River CD male rats were prepared essentially according to Vale *et al.*⁶ and Grant *et al.*⁷. The growth medium was Eagle's minimal essential medium containing 10% foetal calf serum plus standard amounts of glutamine and antibiotics, and the test medium was Earle's balanced salt solution plus antibiotics. After incubation of cell monolayers with and without enterotoxin for 3 h at 37° C, replicate culture fluids were analysed for growth hormone, prolactin, luteinising hormone and thyrotropin by specific double antibody radioimmunoassay. Results were evaluated by analysis of variance, using Dunnett's procedure for multiple comparisons.

Treatment of cell monolayers with concentrations of enterotoxin from 6×10^{-8} M to 6×10^{-9} M significantly increased the basal secretions of all four hormones (Table 1). Enterotoxin concentrations from 6×10^{-10} M to 1.2×10^{-13} M, however, preferentially raised the secretion of growth hormone. In each

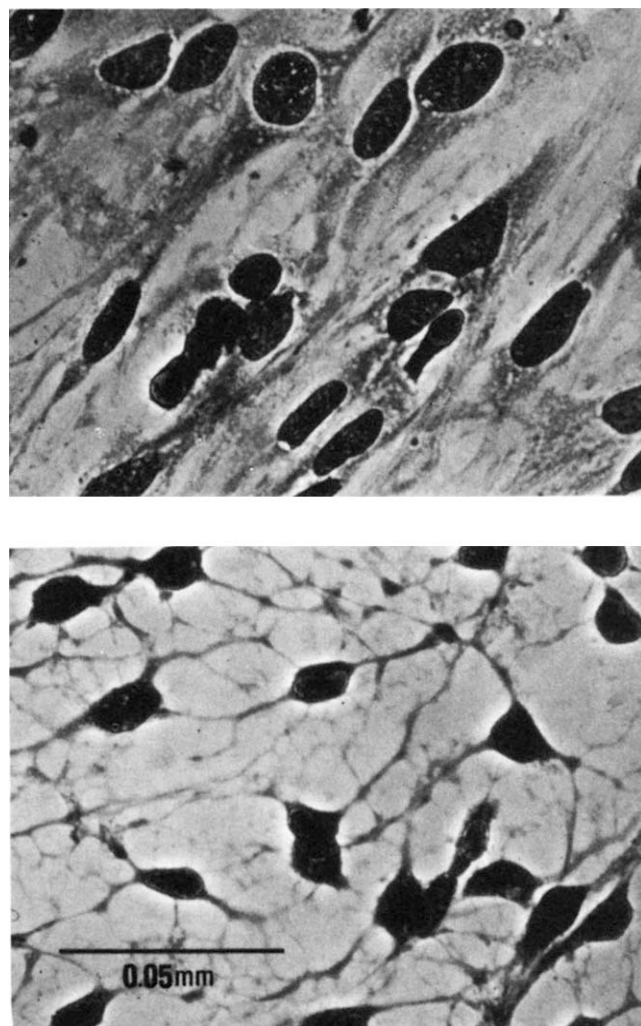


Fig. 1 Monolayer cultures of rat pituitary cells incubated for 3 h at 37° C with test medium (top) or test medium containing 1.2×10^{-11} M of cholera enterotoxin (bottom). Magnification $\times 320$, phase contrast, stained with May-Greewald and Giemsa.)

Table 1 Effect of Concentration of Cholera Enterotoxin on Secretion of Growth Hormone, Prolactin, Luteinising Hormone and Thyroid-stimulating Hormone by Cultured Rat Pituitary Cells

Exp. No.	Cholera enterotoxin concentration (M)	N	Growth hormone	Hormone released (ng ml ⁻¹ , mean $\pm$ s.e.m.)		Thyroid-stimulating hormone
				Prolactin	Luteinising hormone	
1	None	3	436 $\pm$ 20	121 $\pm$ 3	118 $\pm$ 11	505 $\pm$ 89
	6.0 $\times 10^{-8}$	2	543 $\pm$ 44 †	173 $\pm$ 6 *	270 $\pm$ 28 *	1,038 $\pm$ 53 *
2	None	3	153 $\pm$ 9	123 $\pm$ 8	40 $\pm$ 4	52 $\pm$ 17
	6.0 $\times 10^{-9}$	2	510 $\pm$ 26 *	204 $\pm$ 15 *	256 $\pm$ 35 *	179 $\pm$ 37
	6.0 $\times 10^{-10}$	2	484 $\pm$ 18 *	172 $\pm$ 2	49 $\pm$ 6	195 $\pm$ 76
3	None	3	277 $\pm$ 5	174 $\pm$ 7	107 $\pm$ 5	205 $\pm$ 49
	2.4 $\times 10^{-10}$	2	464 $\pm$ 30 *	231 $\pm$ 11 *	122 $\pm$ 5	212 $\pm$ 9
	1.2 $\times 10^{-11}$	2	372 $\pm$ 23 *	195 $\pm$ 4	88 $\pm$ 6	72 $\pm$ 26
4	None	3	288 $\pm$ 10	133 $\pm$ 11	35 $\pm$ 3	NA
	6.0 $\times 10^{-12}$	2	526 $\pm$ 22 *	194 $\pm$ 20	67 $\pm$ 4 *	NA
	1.2 $\times 10^{-12}$	2	371 $\pm$ 9 †	124 $\pm$ 8	67 $\pm$ 2 *	NA
5	None	2	93 $\pm$ 8	88 $\pm$ 8	NA	319 $\pm$ 40
	6.0 $\times 10^{-13}$	2	225 $\pm$ 18 *	87 $\pm$ 6	NA	178 $\pm$ 81
	1.2 $\times 10^{-13}$	2	174 $\pm$ 16 †	81 $\pm$ 5	NA	241 $\pm$ 58
6	None	3	84 $\pm$ 4	97 $\pm$ 7	15 $\pm$ 1	NA
	6.0 $\times 10^{-11}$	3	397 $\pm$ 54 *	131 $\pm$ 5 *	17 $\pm$ 1	NA
	6.0 $\times 10^{-12}$	3	258 $\pm$ 26 †	117 $\pm$ 5	14 $\pm$ 2	NA
	6.0 $\times 10^{-13}$	3	176 $\pm$ 7	109 $\pm$ 3	12 $\pm$ 2	NA

† *P* Compared with control value <0.05.* *P* compared with control value <0.01.

NA, not assayed; N, number of cell culture dishes, each of which was assayed in duplicate.

experiment, the growth hormone response was dose-related. Neither cholera toxoid⁸ at a concentration of 50 μ g ml⁻¹ nor enterotoxin preincubated with specific antitoxin could increase hormone secretion. In addition, cholera toxin, a competitive inhibitor of enterotoxin⁹, and somatostatin, the growth hormone release inhibitory factor¹⁰, each at a concentration of 2.2 $\times 10^{-8}$ M, depressed the release of growth hormone by 1.2 $\times 10^{-11}$ M enterotoxin.

After treatment with enterotoxin, cells remained viable on the basis both of their ability to exclude trypan blue dye and their ability to be subcultured. Further, enterotoxin treatment produced a striking change in morphology: the cells developed elongated cytoplasmic processes which became noticeable within 1 h of exposure to enterotoxin and which, in some cases, seemed to form webs of interconnecting cells (Fig. 1). Replacement of test medium with growth medium alone reversed the morphology to that of the control cultures. Reintroduction of test medium without enterotoxin, however, led to the same morphological transformation which occurred during treatment with enterotoxin, suggesting that the toxin had been irreversibly bound to the cell membrane. In contrast to enterotoxin-treated cultures, monolayers treated with luteinising hormone releasing hormone (LRH) did not exhibit the transformation.

To determine whether cyclic AMP mediated the secretion of growth hormone by the morphologically transformed cells, control cells and cells treated with 1.2 $\times 10^{-11}$ M enterotoxin were collected by trypsinisation and analysed for intracellular cyclic AMP by the method of Gilman¹¹. Enterotoxin-treated cells, secreting four times more growth hormone than control cells, contained approximately twice the intracellular cyclic AMP found in control cells. Cultures more homogeneous with respect to growth hormone-producing cells would probably have an even greater influence of enterotoxin on cyclic AMP content.

In considering the mechanism governing the preferred release of growth hormone at low concentrations of enterotoxin, we compared the kinetics of this release with the kinetics of a well-defined hypothalamic releasing hormone, LRH. The time course of enterotoxin-induced secretion of growth hormone resembled that of LRH-induced secretion of luteinising hormone: significant release of each hormone failed to appear

before 1 h, but for 2 h afterwards both secretions increased linearly with time. This similarity suggests that the sequence of events leading to secretion may be the same.

These findings provide the basis of a sensitive new assay for cholera enterotoxin. The changed morphology of the pituitary cells may in itself offer a rapid and simple means of detection. Moreover, since cholera enterotoxin comprises a large protein marker with specificity for growth hormone-secreting cells, it should facilitate isolation and characterisation of the particular receptors involved.

Taken with the evidence that cholera enterotoxin elevates steroid production in cultured mouse adrenal cells¹², our findings pose the question of whether the toxin may alter host hormone secretion during the course of the disease. Although systemic penetration by enterotoxin has not been demonstrated in clinical or experimental cholera², a peptide fragment of the molecule, small enough to pass through the intestinal mucosa, may retain the minimum structural requirements for stimulating pituitary receptors.

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Note added in proof. We have found recently that enterotoxin from two strains of *E. coli* also stimulates growth with an accompanying morphological transformation.

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Histochemical study of an inhibitor of fibrinolysis in the human arterial wall

THE formation of fibrin is a fundamental biological repair mechanism, but the fibrin has ultimately to be removed by fibrinolysis. This is accomplished by the proteolytic enzyme plasmin, which is converted from plasminogen, an inactive precursor in blood, by an activator. Astrup and Permin¹ have demonstrated that the fibrinolytic activity of tissues is due to such an activator of plasminogen.

To localise fibrinolytic activity in different tissues, Todd² developed a histochemical method in which frozen tissue sections are covered by a thin layer of fibrin rich in plasminogen. During incubation at 37° C, structures containing the plasminogen activator caused lysis which appeared as clear zones in the subsequently stained fibrin. By means of this fibrin slide technique, several investigators^{3,4} found the plasminogen activator in human tissues to be concentrated predominantly in endothelial cells of capillaries and veins.

The endothelial cells of the lumen of human arteries usually showed no activity, however, in contrast to the vasa vasorum of their adventitia. This remarkable lack of activity in endothelial cells of human arterial intima prompted us to carry out a renewed investigation of human muscular and elastic

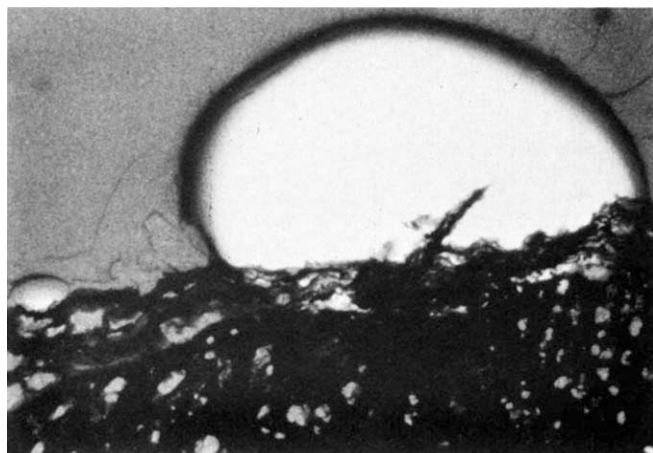


FIG. 1 Lysis produced by the vasa vasorum in the adventitia of the hepatic artery. Left, an initially small zone of lysis; right, an extended zone with flattening towards the media. Harris' alum haematoxylin ($\times 50$).

arteries, as well as veins, by means of the fibrin slide technique. For discrimination between plasminogen activator activity and non-specific protease activity, we prepared slides with bovine fibrinogen with and without plasminogen⁵.

We confirmed the original finding that fibrinolytic activity due to an activator of plasminogen is related to endothelial cells of most veins and capillaries, but not to those of arterial intima. During this investigation, however, our attention was drawn to an outstanding recurrent phenomenon: the initially

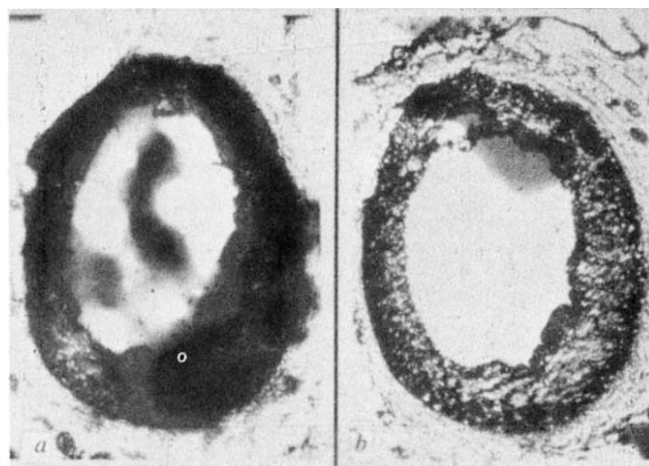


FIG. 2 Cross sections of the splenic artery unheated (a) and heated (b) assayed on plasminogen-free fibrin with the fibrin slide sandwich technique using plasmin as active compounds. After incubation for 140 min, a dark-stained fibrin strand remains on top of the media of the unheated section, while on the heated section all fibrin is lysed. Harris' alum haematoxylin ($\times 5$).

small round areas of lysis due to plasminogen activator in the vasa vasorum of the arterial adventitia flatten towards the site of the media, while they extend during prolonged incubation. Consequently, their centres shift to lateral (Fig. 1). This feature was more pronounced in human muscular than in elastic arteries, while the distortion of lytic zones was hardly noticeable around large human veins. A possible explanation for this could be that medial tissue contains an inhibitor of fibrinolysis which may diffuse into the fibrin layer above it, thus preventing lysis at those sites.

Evidence for this hypothesis was given by demonstrating the inhibition of fibrinolytically active compounds placed on top of the medial site of human arteries. This was achieved by modifying the fibrin slide technique as follows: frozen sections of the tissue to be examined were placed on a microscope slide, covered by fibrin, and left for at least 2 h in a moist refrigerator, to allow diffusion of inhibiting components from the sections into the fibrin layer. Fibrinolytically active frozen sections were then placed on top of the fibrin film after which the preparations were incubated for various lengths of time at 37° C, fixed, and stained. We called this technique the 'fibrin slide sandwich technique'⁶. When used on fibrin films rich in plasminogen, the fibrinolytically active layer of the 'sandwich' consisted of a frozen section of human lung known to be rich in plasminogen activator, or a section of a frozen urokinase solution (human urokinase, Leo Pharmaceuticals, Copenhagen, dissolved to 7 Ploug units ml⁻¹ in saline barbital buffer with 15% gelatin). Human plasmin⁷ (Michigan Department of Public Health, Michigan, diluted to 1.5 U ml⁻¹ in saline with 15% gelatin) was used on plasminogen-free fibrin.

By means of the sandwich technique using both activator and plasmin as the active layer, a clear inhibition of fibrinolysis was demonstrated in the medial area of fresh human arteries but not in the adventitial region. This inhibitory effect was indicated by a dark-stained fibrin strand remaining on top of the media while the active top layer had lysed most of the fibrin which it contacted. Usually some fibrin is observed remaining in the lumen at the moment when the fibrin adjacent to the adventitia is already lysed (Fig. 2a). Inhibition of both activator and plasmin by human elastic arteries was not as effective as by muscular ones; the walls of large veins, for example the superior vena cava, showed very little inhibition.

During further attempts to confirm our evidence, it was discovered that heating tissue sections in a dry oven at

100° C for 12 h before covering with fibrin abolished the inhibition effect. The absence of a dark-stained fibrin strand on top of the media of a heated section (Fig. 2b) shows the loss of inhibition in contrast to its presence in fresh sections (Fig. 2a).

Our results thus indicate the presence of an inhibitor of fibrinolysis in the human arterial wall which is able to diffuse into fibrin. This provides a natural explanation for the distortion of lytic zones to the medial site, and possibly for the absence of fibrinolytic activity in the arterial intima as seen with the fibrin slide technique. The physiological significance of this inhibitor in human arteries remains to be discovered, but its presence should be kept in mind when considering the role of fibrinolysis in vascular disease.

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Temperature-sensitive Mammalian Cell Line Blocked in Mitosis

SEVERAL temperature-sensitive mammalian cell mutants in culture have been isolated¹⁻¹⁰, including three defective in protein syntheses⁶, cytokinesis⁵ and the processing of ribosomal RNA¹⁰, respectively. I have now isolated a hamster cell mutant in which mitosis is blocked in metaphase.

Hamster cells HM-1 from Kam Laboratories were grown in Dulbecco's modified Eagle's minimal essential medium (MEM) supplemented with 10% calf serum (Grand Island Biological

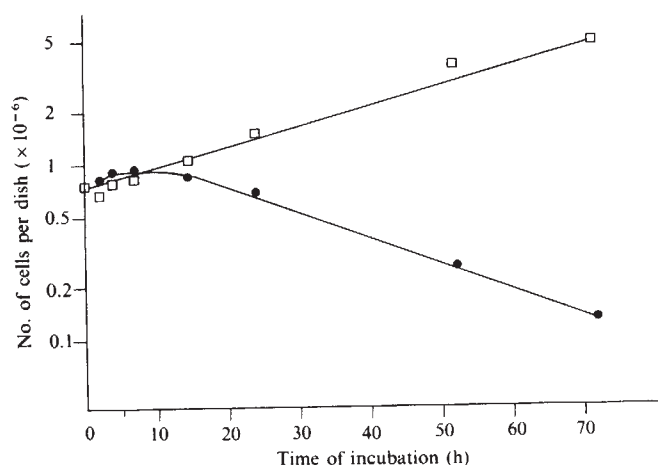


Fig. 1 Growth of ts-546 at 33° and 39° C. Falcon 60-mm culture dishes were inoculated with cells and incubated at 33° C for 3 d. Half the dishes were transferred to 39° C. The medium in two dishes was removed, trypsin added and cell number counted at the time of transfer (time 0) and at intervals thereafter. □, Cells at 33° C; ●, at 39° C.

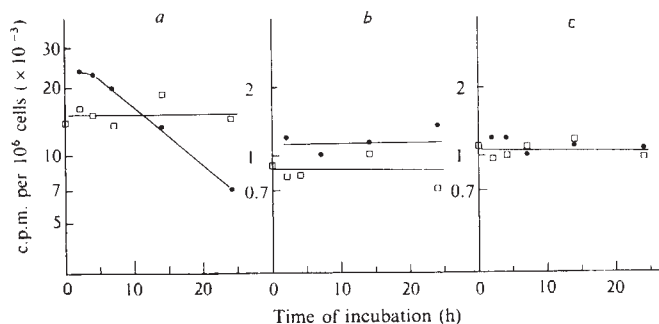


Fig. 2 Incorporation of labelled precursors by ts-546 cells. All the steps were carried out in warm rooms with temperature fluctuation controlled within $\pm 0.05^\circ$ C. 1×10^5 cells were inoculated into each 60-mm dish and incubated for 72 h at 33° C permitting the cells to reach log-phase growth at a concentration of about 1×10^6 cells per dish. At time 0, half of the dishes were transferred to 39° C. Two dishes each at the time of transfer and intervals thereafter were labelled with $0.5 \mu\text{Ci ml}^{-1}$ ^3H -thymidine (a), $1 \mu\text{Ci ml}^{-1}$ ^3H -uridine (b) or $0.5 \mu\text{Ci ml}^{-1}$ ^3H -L-leucine (c) for 30 min at 33 and 39° C. The medium was then removed and 10% trichloroacetic acid added. Acid insoluble material was collected by filtration and radioactivity was counted in a Beckman scintillation counter. □, Cells at 33° C; ●, at 39° C.

Co.) without added antibiotics. To isolate the mutants, 33° C was used as the permissive temperature and 39° C the non-permissive temperature. Cells were treated with 10^{-5} M N-methyl-N'-nitro-N-nitrosoguanidine (NG) which killed approximately 70% of them. The rest were grown at 33° C and added to 60-mm Falcon plastic culture dishes at a concentration of 2×10^5 per dish and incubated at 39° C for 24 h, at which time $10 \mu\text{g ml}^{-1}$ cytosine arabinoside and $10 \mu\text{g ml}^{-1}$ 5-fluorodeoxyuridine were added. The dishes also received $40 \mu\text{g ml}^{-1}$ uridine to prevent 5-fluorouracil, a breakdown product of 5-fluorodeoxyuridine, from interfering with RNA synthesis¹¹. After a further 24 h at 39° C, the medium was changed to MEM and the dishes were returned to 33° C. When clones had developed, the cells were transferred to Linbro 24-well multi-dishes. Half the cells in each clone were added to wells in dishes incubated at 33° C and half were added to the corresponding wells in dishes incubated at 39° C. The cells which grew at 33° C but not at 39° C were removed and further tested for growth at 33° and 39° C. No mutants have been isolated without NG treatment. Of more than fifty mutants isolated, one, ts-546, was found in which mitosis is blocked in metaphase at the non-permissive temperature.

The wild-type cell differed slightly from the mutant in growth rate at 33° C. The parental HM-1 cells grew at 33° and 39° C with doubling times of 20 and 12 h respectively. Mutant ts-546 grew well at 33° C with a slightly longer doubling time of 25 h (Fig. 1). When the ts-546 mutant cells were placed at 39° C, the cell number increased slightly for 2-4 h and then declined (Fig. 1). The decrease in cell number was probably a result of the cell counting procedure used, in which medium was removed before trypsin was placed in the dishes to dislodge the cells attached to the bottom. The mutant ts-546 cells arrested in mitosis (described later) tend to be loosely attached or not attached and were readily removed with the medium. No cells remained attached to the dishes after 5 d.

When 50 or 100 cells were plated per 60-mm dish, the parental wild type cells plated with $>90\%$ efficiency at 33° and 39° C (Table 1). The plating efficiency of the mutant was slightly reduced at 33° C, but at 39° C, when up to 2×10^6 ts-546 cells were plated per 60-mm dish, no clone was found (Table 1). Cell killing at 39° C was thus independent of the density of cells inoculated. Of the 10^8 cells cultured at 39° C, two revertants have been found giving a reversion frequency of about 5×10^{-7} .

Incorporation of labelled precursors into acid-insoluble material in wild type and ts-546 cells was also measured. A sample of 10^5 cells was inoculated per 60-mm dish and grown for 72 h until log-phase growth was reached at approximately 10^6 cells per dish. Half the dishes were then switched to 39°C . At intervals, ^3H -thymidine, ^3H -uridine or ^3H -L-leucine was added for 30 min at both temperatures. Incorporation of ^3H -thymidine per cell began to decrease after 4 h for the cells incubated at 39°C (Fig. 2). The decrease could be partly or totally due to the fact that as the cells entered mitosis, they were arrested in metaphase (described later) and consequently unable to enter G1 and re-initiate DNA synthesis. No change in the incorporation of the labelled material into RNA or proteins was found at 39°C (Fig. 2). The incorporation procedure eliminated any loosely attached or floating cells when the medium was removed before trichloroacetic acid was added. This may explain why RNA synthesis per cell was not decreased even though a fraction of the cells was arrested in mitosis and expected to synthesise little or no RNA¹².

Mitotic figures were examined after the mutant cells were grown at 33°C and 39°C . When the mutant cells were grown at 33°C , normal mitotic figures were observed (Fig. 3a). After the cells were shifted to 39°C , many aberrant mitotic figures appeared (Fig. 3b). The chromosomes seemed no longer to be held together by the spindle. They were scattered as in colchicine-induced 'colchicine-metaphase'¹³ (C-metaphase), where spindle fibre mechanisms are partially or totally destroyed, and the chromosomes lose their normal orientation. To distinguish the figures seen here from C-metaphase, the aberrant mitotic

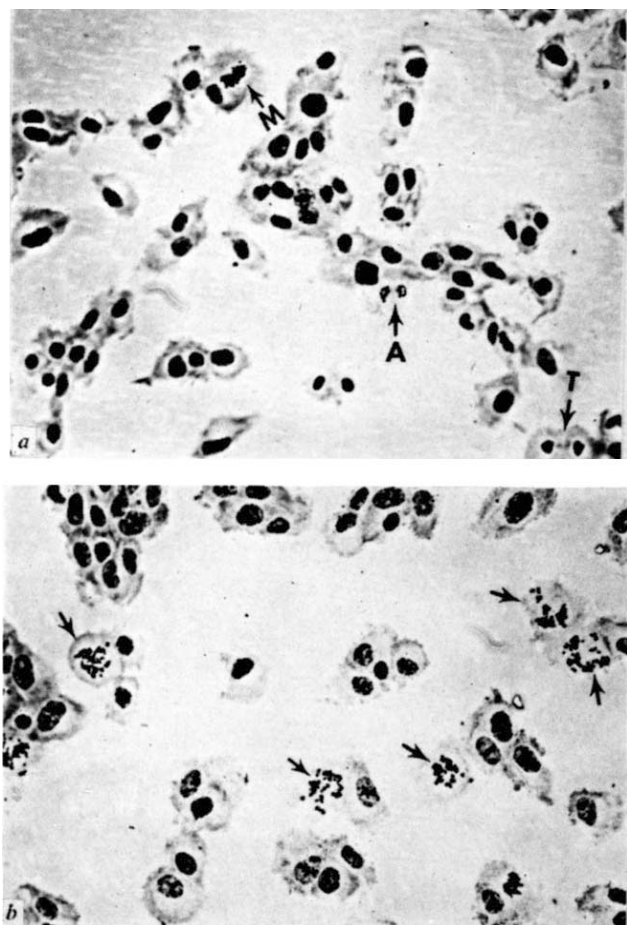


Fig. 3 ts-546 cells at 33°C . Cells grown at 33°C were washed with phosphate-buffered saline, fixed with 3 : 1 methanol-acetic acid and stained with aceto-orcin. Arrows point to normal metaphase (M), anaphase (A) and telophase (T) in (a) and aberrant ts-546-metaphases in (b). ($\times 200$.)

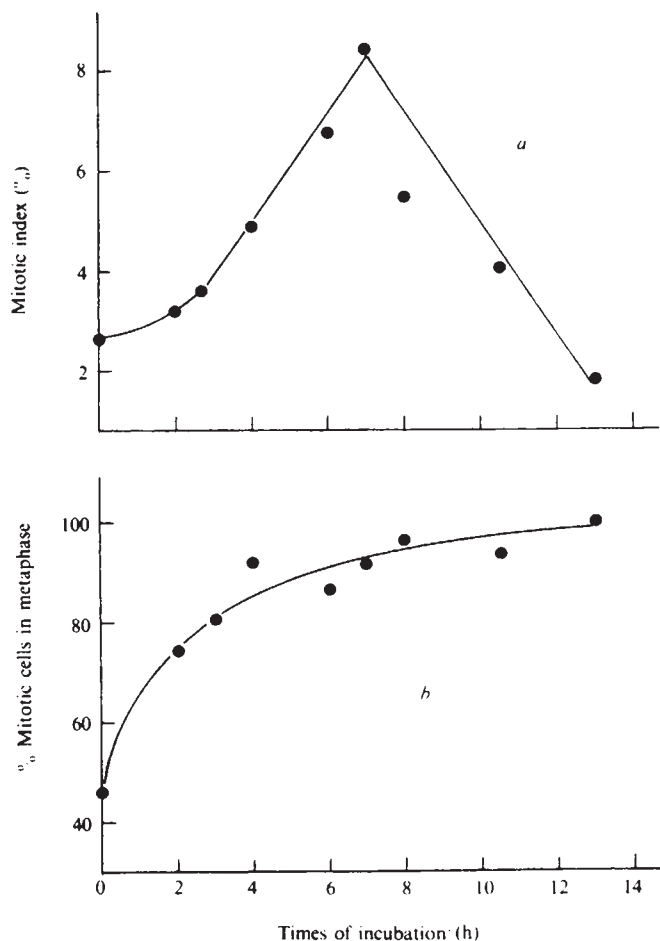


Fig. 4 Change in mitotic index and percentage of mitotic cells in metaphase in ts-546 cells after incubation at 39°C . 10^6 cells in log-phase growth per 60-mm dishes were transferred to 39°C at time 0. Cells were fixed and stained at intervals. The mitotic index (a) is expressed as the percentage of cells in mitosis. The percentage of mitotic cells in metaphase (b) is defined as

$$\frac{\text{No. of cells in metaphase and ts-546 metaphase}}{\text{No. of mitotic cells}} \times 100.$$

figures induced by temperature shift are termed 'ts-546-metaphase'.

Immediately after the cells were switched to 39°C , mitotic figures (metaphase, ts-546-metaphase, anaphase plus telophase) started to accumulate. The mitotic index, or the fraction of cells in mitosis, increased from approximately 3% to more than 8% after 7 h at 39°C and decreased thereafter (Fig. 4a). The fact that the mitotic index did not increase beyond 8% was most likely due to the fixation procedure with loosely attached or floating cells lost during washing. Time-lapse photomicro-

Table 1 Clone forming ability of parental (HM-1) and mutant (ts-546) cells at 33°C and 39°C

No. of cells plated	No. of clones formed			
	33°C		39°C	
	HM-1	ts-546	HM-1	ts-546
50	46.5	—	54.5	0
100	92.5	48.0	—	0
200	—	89.5	—	0
1,000	—	—	TMTC	0
10,000	—	—	TMTC	0
10^5	—	—	TMTC	0
2×10^6	—	—	TMTC	0

Cells were plated in 60-mm dishes and incubated. After 7 d at 39°C or 10 d at 33°C , cells were stained with haematoxylin and the clones counted.

TMTC, too many to count.

graphs demonstrated that, in a culture of ts-546 cells at 39° C, the number of round, refractile, mitotic-like cells increased continuously and subsequently floated into the medium. When a culture of ts-546 cells was incubated at 39° C for 24 h, at least 50% of the cells appeared to be rounded and mitotic-like. At the same time, few dumbbell-like figures characteristic of anaphase or telophase cells were seen (Fig. 5).

The number of metaphases and ts-metaphases increased from approximately 50% of the mitotic figures at the time of temperature switch to close to 100% 8 h later (Fig. 4b). The number of anaphases and telophases correspondingly decreased from 50% to nearly 0.

The results show that when hamster ts-546 mutant cells were switched from 33° to 39° C, the cells ceased to divide almost immediately. With further incubation at 39° C, cells were killed regardless of their density. Reversion frequency was about 5×10^{-7} . No generalised defect in DNA, RNA or protein synthesis was found at the non-permissive temperature. At 39° C the mitotic cells were arrested in metaphase and ts-metaphase, which is seen as a failure of the normal spindle fibre mechanism to hold the chromosomes in their normal metaphase orientation. The ts-metaphase figures, under a light microscope, appear to be similar to the c-metaphases resulting from colchicine treatment.

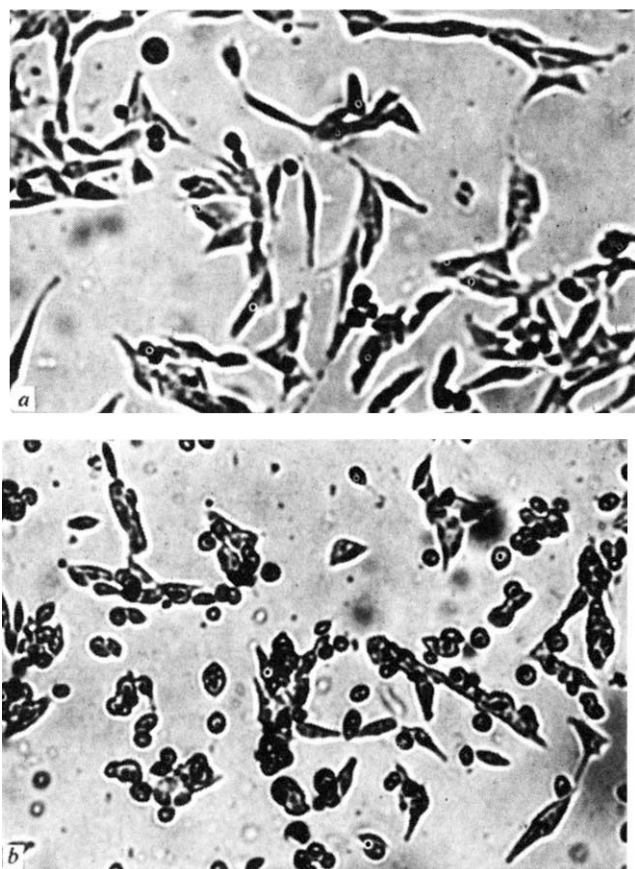


Fig. 5 Accumulation of mitotic-like cells at 39° C. Cells in dishes in log-phase grown at 33° C were incubated at 39° C for 24 h when the photograph was taken. ($\times 140$.)

Since mitotic cells generally synthesize very little or no RNA¹², one may ask that why is no reduction of RNA synthesis observed at 39° C? The cells used for measuring ³H-uridine incorporation were those which remained attached to the dishes where the maximum number of mitotic cells reached only 8% after shifting to 39° C (Fig. 4a). Consequently 92% or more of the cells used in the incorporation experiment were non-mitotic. The finding that the cells not in mitosis incorporate

a normal amount of ³H-uridine is thus compatible with the conclusion that the ts-546 cells in mitosis are blocked in metaphase at the non-permissive temperature.

Several mechanisms might account for the accumulation of mitotic cells in metaphase and ts-metaphase at 39° C. The gene controlling the production of spindle fibre proteins or microtubules could have suffered a mutation resulting in defectiveness and inability of the proteins or microtubules to orient properly at 38° C. A second possibility is that a protein holding the spindles together became defective. A third alternative is that a protein holds the spindle proteins and chromosomes together, becoming defective at the non-permissive temperature, so that the chromosomes are disengaged from the spindles. It is also conceivable that the defective protein, when normal, binds the spindles to the centriole. There is also the possibility that the synthesis of the spindle fibre proteins or other factors involved in karyokinesis failed to take place at 39° C. These possibilities are being investigated.

It is to be hoped that the isolation of temperature sensitive mutants defective in mitosis could lead to the identification of factors which are essential in mitosis. It might also be possible to identify the time during the cell cycle when these factors are synthesised. Furthermore, the possibility that mitosis can be arrested because specific factors become defective at a higher, non-permissive temperature may provide methods of arresting metaphase and controlling cell growth by specific inhibition of these factors.

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Facilitation of Learned Resistance to Audiogenic Seizures in Balb/cCrgl Mice by *d*-LSD-25

ALTHOUGH conditioning techniques applied to electrophysiological behaviour are presently being used in behaviour therapy for human epilepsy^{1,2}, complete cure is still a hope of the future. Extensive reports indicate that minute amounts of *d*-lysergic acid diethyl amide (*d*-LSD-25) could be used to assist current biofeedback training and other therapies used to effect change in the EEG and seizure susceptibility of

epileptics⁸ and we note many studies of the facilitation effect of small doses of *d*-LSD-25 on learning and memory¹³.

Several methods, including many behavioural ones¹⁴⁻¹⁸, are known for changing audiogenic seizure susceptibility in inbred mice. Elaboration of a conditioned response is known to interfere with the occurrence of these seizures¹⁹ and exploratory activity and learning ability are positively correlated with seizure resistance in rodents²⁰, as are several factors related to stress^{14,21}. We confirmed these effects in experiments with mice in which the administration of 3.65 μ g of *d*-LSD-25, 30 min before conditioning, significantly facilitated long-term habituation and also conditioned inhibition of audiogenic seizures induced by an intense, high-frequency sound presented 1 d after the end of training.

Long-term habituation was brought about by exposing the mice to a subseizure threshold tone 1-4 d before challenging them to seize with the louder tone. This effect is related to the classical 48 h refractory period found after intense stimulation¹⁴. Conditioned inhibition of seizures was accomplished by pairing a light with brief subthreshold tones, and subsequently presenting the light 5 s before the challenge stimulus on test night.

The use of multiple exposures to improve estimates of seizure susceptibility was first reported by Fuller and Sjursten²². Henry²³ later showed that a priming stimulation of only 30 s at an early age induced development of seizure susceptibility which peaked 4-5 d later, suggesting a method of more reliable control of seizure susceptibility. In standardising procedures for Balb/cCrgl mice, we found that peak seizure incidence (88%) on day 26 after priming on day 21 was equal to the cumulative seizure incidence computed as the percentage of mice that seized at least once during four consecutive exposures on days 21, 28, 35 and 42. In our experiments, the treatment variables were introduced between days 21 and 26.

Six hundred and eighty-five mice were randomly assigned to groups by split litter design, weighed and used to test the effects of pure *d*-LSD-25 (Sandoz) given intraperitoneally (7.3 μ g ml⁻¹; average dosage 180 μ g kg⁻¹; standard dose, 3.65 μ g per mouse) on the alteration of audiogenic seizure susceptibility by experimental and control procedures which are listed below. All mice were primed on day 21 (1900 h) and tested on day 26 (1900 h). The main experimental procedures were as follows. Series 1, running to escape from the hand of the experimenter in an activity wheel at 50 m d⁻¹ on days 22-26 (1900 h); series 2, the direct effect of *d*-LSD-25 given on days 21 and 26 (30 min before priming and testing respectively) as compared with the same injected only on days 24 and 25 (1900 h); series 3, the effect of *d*-LSD-25 given 30 min before training on long-term habituation on test night induced by five 1 min alternating exposures to a subseizure threshold 10 kHz pure tone (60 dB above 0.0002 dynes cm⁻² presented repeatedly on days 22-25 (1200 h) as compared with the effect when injected 30 min before receiving 20 min continuous exposure on days 24 and 25 (1200 h); series 4, the effect of *d*-LSD-25 injected 30 min before a 15 min non-random classical conditioning session of delayed light (25 W red, diffuse bulb)-tone (10 kHz at 60 dB above 0.0002 dynes cm⁻²) pairing (ISI, 1.5 s, US, 10 s, time-out, 15 s) on day 25 (1900 h). On day 26 the conditioned stimulus (red light) was presented 5 s before the challenge stimulus (condition CS in series 4 of Table 1).

Drug controls received equal amounts of handling and of the diluent (0.9% sterile saline) and yoke stimulation. Additional control groups were included as indicated in Table 1. For series 1, controls received equal amounts of exposure to the hand of the experimenter in a fixed activity wheel, in series 2, controls were not primed, and in series 3 they received equal exposure to the experimental chamber. For series 4, experiment 1, controls received equal cyclic exposure to light-only and tone-only. Controls in experiment 2 of series 4 received identical light-tone pairing but were not

Table 1 Effect of *d*-LSD-25 and learned resistance to audiogenic seizures in Balb/cCrgl mice

Series	Experiment	Condition	Drug	% Seized	N	adj. χ^2
1		Activity wheel training				
		Run	—	62	37	
		Yoke	—	86	36	
		Total			73	4.60*
2		Direct action of LSD				
	1	Primed	LSD	88	16	
			Saline	75	16	
		Unprimed	LSD	0	16	
			Saline	0	16	
		Total			64	0.59
	2	Primed	LSD	84	25	
			Saline	92	25	
		Total			50	0.09
3		Habituation and LSD				
	1	Tone	LSD	50	50	
			Saline	84	50	
		Controls	LSD	90	10	
			Saline	90	10	
		Total			120	13.02†
	2	Tone	LSD	0	20	
			Saline	25	20	
		Controls	LSD	65	20	
			Saline	85	20	
		Total			80	27.73†
4		Conditioned inhibition and LSD ‡				
	1	Light-tone	LSD	41	37	
			Saline	60	37	
		Light-only	LSD	66	37	
			Saline	66	37	
		Tone-only	LSD	60	25	
			Saline	60	25	
		Total			198	6.19
	2	CS	LSD	28	25	
			Saline	44	25	
		CS	LSD	52	25	
			Saline	56	25	
		Total			100	4.43

* $P < 0.05$.

† $P < 0.001$.

‡ LSD, light-tone treatment is significantly different from remaining control groups pooled in this series (adj. $\chi^2 = 5.31$, d.f. = 1, $P < 0.05$).

exposed to the conditioned stimulus on day 26 during challenging (Conditions CS).

All mice were primed and tested with a 90 s 10 kHz pure tone at 100 dB above 0.0002 dynes cm⁻². A concentration check after 6 months showed no change in the LSD solution kept in the dark, near room temperature.

The results are summarised in Table 1. Repeated locomotor practice significantly reduce seizure susceptibility by 24%. This effect is not strong and was not significant in a smaller sample (data included in experiment 1 of series 3) treated with *d*-LSD-25. The effect is not due to a single session of running just before testing on day 26. Earlier work with activity wheel stress and audiogenic seizures found very similar results¹⁵.

At 3.65 μ g per mouse, repeated or single doses of *d*-LSD-25 had no direct effect upon seizure susceptibility. Only one 3.65 μ g dose, however, increased conditioned inhibition and long-term habituation of audiogenic seizures in the presence of the conditioned-unconditioned and unconditioned stimulus respectively. The increase in habituation was more significant. Future work on conditioned inhibition might use more elaborate procedures to study the effects of *d*-LSD-25 on higher learning.

These results did not depend on *d*-LSD-25 being present during testing performed 24 h after injection and training. Controls showed learning occurring to a lesser degree independent of the *d*-LSD-25 state during acquisition. These results support earlier findings that low amounts of *d*-LSD-25 facilitate ongoing processes in the brain, affecting learning and memory⁴⁻¹³. Studies of the best time relationships between states and of the use of equal or preferably smaller doses to effect reverse tolerance are needed. The symptoms of overdose are well known to have effects opposite to those of lower doses³.

Mice exhibit the highest resistance to high-dose effects of *d*-LSD-25, as much as 300 µg per mouse being required to produce overt changes in its behaviour²⁴. We have shown here that as little as 1% of this dose caused significant facilitation of learned behaviour which reduced subsequent seizure susceptibility. Although caution must be observed²⁵ these data may be extrapolated to the human condition on at least three grounds. Similar forms of reflex epilepsy exist between the two species (for example, startle, audiogenic and musicogenic epilepsy²⁶⁻²⁹). Acoustic stimulation has been found to be superior to intermittent photic stimulation or hyperventilation in defining the cortical paroxysmal focus in the EEG of humans with temporal lobe epilepsy³⁰. (There is reason to believe that a cortical focus facilitates the audiogenic seizure in mice.) Also, seizures in both species are subject to the effects of learning^{1-3,19}.

The facilitation effect of single low doses on learning has been reported for humans as well as several animal species in many different situations. Suggestions that *d*-LSD-25 causes undesirable side-effects, such as chromosomal damage, when used in clinically useful doses, have been refuted by investigations *in vitro* and *in vivo* in several species including humans^{3,31-37}. As neural degeneration and functional depression due to anticonvulsant medication are a problem³⁸⁻⁴⁰, human experimentation with low dose schedules and learning should be considered.

By comparing dose-effect data from the mouse and human it is possible to estimate the optimum dosage required to facilitate learning and memory in the human. The mouse detoxifies *d*-LSD-25 ten times faster, has a metabolic rate ten times greater and a much lower brain/body weight ratio than humans; so its high resistance to direct effects is not surprising. Overdose symptoms peak at approximately 300 µg for the mouse²⁴ and 1,000 µg for the human⁴¹. As 1% of this dose significantly reduced seizure susceptibility in mice through learning, the optimum dose facilitating condition inhibition of seizures in human epileptics could be as low as 10 µg or less (that is, about 0.1 µg kg⁻¹ as compared with the so-called moderate dosage of 1-2 µg kg⁻¹). Further, since 100 µg represents the anticonvulsant threshold in mice, at least 300 µg should be required to suppress motor seizures directly in the human epileptic.

The physiological mechanisms underlying the effect of *d*-LSD-25 on brain function are relatively well understood³. It acts directly as an anticonvulsant when taken in high doses. Approximately 1-5 mg kg⁻¹ must be given to the mouse to increase seizure resistance independent of conditioning^{42,43}. This direct protection is related to the facilitation of motor and sympathetic nervous activity within this dose range for the mouse^{42,44-47}. High dose inhibition of synaptic transmission through the thalamus⁴⁸ is probably not a necessary part of the anticonvulsant action in mice, since all brain rostral to the inferior colliculus is unnecessary for elaboration of the audiogenic seizure sequence⁴⁹, but may be where subcortical activation of a cortical focus is necessary for the seizure to occur^{50,51}.

Bradley and coworkers⁵² showed that small amounts of *d*-LSD-25 amplify the ongoing effects of sensory collateral inputs to the reticular formation of the brainstem without directly affecting sensory input to the cortex⁵³ or directly exciting the output cell core⁵⁴ of the reticular formation. They later discovered that these effects focused attention of

the animal on to a neutral or habituated stimulus to the degree found with conditioned stimuli (where response thresholds were not affected by the dosage used) without directly arousing the animal or preventing its sleep. They suggested that in small amounts, *d*-LSD-25 increases the significance level of sensory stimuli by facilitating mechanisms which underlie attention and block short-term habituation. The inhibitory effects of attentional mechanisms in animal and human epileptics are extensively documented although they are still not fully understood⁵⁵.

Amphetamine has also been shown to facilitate learning and memory only within an optimal dose range⁵⁶ and also to reduce audiogenic seizure susceptibility at high doses^{21,42,43}. An optimal range in the dose-effect curve is a classical finding. Some drugs that facilitate learning at low doses, however, act as poisons and convulsants at higher ones (for example, strychnine)⁵⁶. Drugs chosen to facilitate behaviour therapy for epilepsy could conveniently act as anticonvulsants at high or cumulative doses while simultaneously minimising side-effects.

A recent review⁵⁷ re-affirmed past conclusions that LSD can be a safe therapeutic instrument when properly administered to suitable subjects in controlled settings. Tetrahydrocannabinol^{58,59,60} and psilocybin^{50,51} also act as anticonvulsants. This combination of facilitation of learning and memory in minute doses, direct anticonvulsant action in the upper dose range, reverse tolerance with proper scheduling, and the absence of addiction and proven side effects directly attributable to the drugs, suggests the use of psychedelic compounds as effective and safe adjuncts to current behavioural and electrophysiological biofeedback procedures presently used to treat epilepsy and a variety of other illnesses.

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without the actual formation of a covalent bond⁴. It is surprising that antibody could act as a carrier for chlorambucil under these conditions, as has been proposed⁵, as the two molecules would be expected to dissociate in the *in vivo* environment. It is, therefore, probable that the increased cytotoxicity of chlorambucil in the presence of antibody is attributable to some other mechanism. The experiments described here are an attempt to elucidate this problem further.

The target cells for these experiments were polyoma-transformed BHK21/C13 cells (J_1) grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum which had been heated to 56° C for 30 min to inactivate complement. The cells were collected using a Tris-buffered saline containing 0.02% versene. An antiserum to these cells was produced in New Zealand white rabbits. Each rabbit received approximately 1×10^8 intact cells intramuscularly twice weekly for 4 week. The resulting antiserum contained antibodies for surface components of J_1 cells as shown by membrane immunofluorescence by the indirect method using fluorescein-labelled sheep antirabbit globulin (Miles-Serevac). The antiserum was also cytotoxic for J_1 cells in the presence of guinea pig complement (Wellcome) as shown by isotope release from cells prelabelled with radioactive chromium (^{51}Cr); 50% of cells were lysed at a 1 : 320 dilution. Control normal rabbit serum (NRS) was obtained by bleeding the rabbits before immunisation. Both the NRS and the immune serum (IRS) were heat inactivated, sterilised through a 0.22 μ Millipore filter and stored

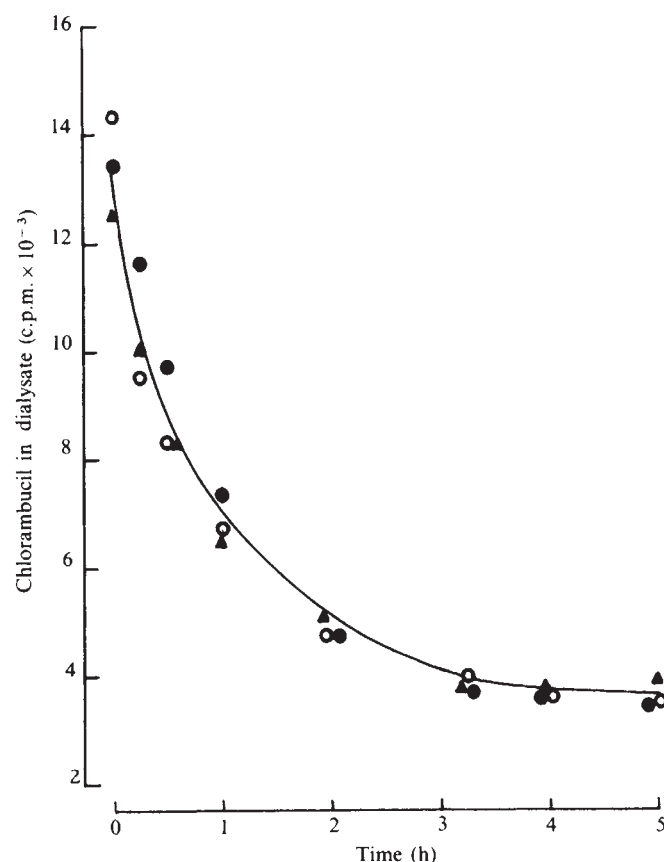


Fig. 1 10 ml volumes of undiluted calf serum were placed in lengths of Visking dialysis tubing (0.25 inch diameter), which were then arranged as flat coils and covered with 10 ml of one of the following solutions: (1) 1×10^{-6} M ^3H -chlorambucil (specific activity 118 mCi mmol⁻¹) in PBS (○); (2) rabbit immunoglobulin (IgG) (Miles-Serevac) 50 $\mu\text{g ml}^{-1}$ with 1×10^{-6} M ^3H -chlorambucil preincubated on ice for 15 min (▲); (3) bovine serum albumin 50 $\mu\text{g ml}^{-1}$ with 1×10^{-6} M ^3H -chlorambucil preincubated on ice for 15 min (●). Dialysis proceeded at 37° C and 100 μl samples of the dialysate were taken at timed intervals for scintillation counting.

Augmentation of Cytotoxic Drug Action by Antibodies Directed at Cell Surface

THE concept of attaching cytotoxic agents to tumour-specific antibodies in the treatment of cancer is therapeutically attractive. In this way the specificity of the agents would be increased and their systemic toxicity reduced. This immunochemotherapeutic approach has been successfully applied in experimental systems when the cytotoxic agent has been covalently bound to the antibody¹⁻³. Similar success has been reported with the nitrogen mustard chlorambucil, merely physically adsorbed on to the globulin fraction from tumour-specific antiserum

at -20°C until used. Chlorambucil BP (Wellcome) was prepared just before use by dissolving a few mg in 0.5 ml of 0.5 M sodium bicarbonate, at room temperature and then immediately diluting in cold phosphate-buffered saline solution (PBS), pH 7.2.

the possibility that the increased sensitivity of the cells to chlorambucil was due to an increased rate of mitosis; (3) since it has been shown that chlorambucil in high concentrations ($6 \times 10^{-3}\text{ M}$) causes lysis of red blood cells⁷, the possibility that IRS may allow lysis of J_1 cells by lower concentrations of

Table 1 Cloning efficiency of Py BHK(J_1) cells treated with combinations of C, NRS and IRS described in text

Means $\pm$ standard deviations of colonies in quadruplicate cultures.									
PBS	C	NRS	NRS/C	NRS-C	NRS+C	IRS	IRS/C	IRS-C	IRS+C
50 ± 2.2	38 ± 5.7	64 ± 6.8	39 ± 6.1	59 ± 8.7	39 ± 10	53 ± 16.5	14 ± 7.8	58 ± 6.8	12 ± 11

Differences: NRS/C and IRS/C, $P < 0.01$; NRS+C and IRS+C, $P < 0.02$; IRS/C and IRS+C, not significant.

The effect of chlorambucil on J_1 cells in the presence of immune serum was studied using a colony formation inhibition assay. One hundred cells were plated in 50 mm plastic Petri dishes in a 4.5 ml volume of medium with 10% inactivated calf serum. Then the cultures received one of the following additions: (1) 0.5 ml of PBS alone or containing one of the following substances: chlorambucil (C) to give a final concentration in the culture medium of $6 \times 10^{-6}\text{ M}$, or either NRS or IRS at a final dilution of 1:80 in the culture; (2) 0.5 ml of PBS with NRS or IRS mixed with C in the cold so that only physical adsorption of the drug to protein but little or no covalent reactions would occur⁶ (NRS/C and IRS/C); (3) 0.5 ml of PBS with NRS or IRS mixed with C and preincubated at 37°C for 24 h to allow complete reaction of the C, mainly alkylation of protein but also some hydrolysis⁶ (NRS-C and IRS-C); (4) NRS or IRS in 0.25 ml of PBS and C in 0.25 ml PBS added at the same time but separately to the dishes (NRS+C and IRS+C). In all cases the final concentration of chlorambucil and the dilution of NRS or IRS was the same. After incubating the dishes in humidified incubators flushed with carbon dioxide for 8 d, the medium was aspirated, the colonies were washed once with PBS, fixed with methanol, stained with 20% Giemsa stain and counted.

The results (Table 1) show that the cloning efficiency was the same with PBS, NRS alone, IRS alone, NRS-C and IRS-C. In those dishes that received active chlorambucil, namely C, NRS/C and NRS+C, there was a small reduction in the number of colonies. The reduction was however much greater when the drug was in the presence of IRS (IRS/C and IRS+C). Preincubation of IRS and chlorambucil in order to favour the adsorption of the drug on to antibody was not a prerequisite for augmenting the cytostatic effect of the drug.

These results corroborate, in a different system, those of Ghose *et al.*⁵ but do not support the hypothesis that they occur because the antibody acts as a carrier for chlorambucil. In fact, the identity of the effect of IRS/C and IRS+C would then require that chlorambucil associates preferentially with and binds strongly to rabbit immunoglobulins in competition with the calf serum proteins present in the medium. That this is not the case was shown by the experiment described in Fig. 1. The results show that the rate of disappearance of chlorambucil from the dialysate is the same whether or not it contains rabbit immunoglobulin, thus excluding preferential binding of chlorambucil to the immunoglobulin.

The mechanism whereby antibodies directed at the cell surface increase the susceptibility of polyoma-transformed BHK21/C13 cells (J_1) to the cytostatic effect of chlorambucil remains unknown. Several possibilities were ruled out by the following results. (1) The uptake of tritiated-chlorambucil by IRS-treated cells was not different from that of NRS-treated cells; (2) the mitotic rate of cells in the presence of IRS or NRS as determined by growth curves was the same, thus excluding

chlorambucil was considered. Cells were prelabelled with ^{51}Cr , treated with IRS or NRS together with graded doses of chlorambucil, and ^{51}Cr release was measured. While it was confirmed that these cells were lysed by $6 \times 10^{-3}\text{ M}$ chlorambucil too, there was no lysis in lower concentrations with either IRS or NRS.

Experiments using other cell systems and agents are now in progress to test the generality of this augmenting effect of antibodies on cytotoxic drug action and its possible use in cancer therapy. It is known that when tumours are associated with a well developed humoral immune response, for example in Burkitt's lymphoma⁸ and choriocarcinoma⁹, the response to cytotoxic drugs is often dramatic. We speculate on the basis of the experiments described above that in these malignant diseases it may be the binding of antibody to the tumour cells that by some means, not yet determined, is responsible for their marked susceptibility to chemotherapy.

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Spatial Heterogeneity and Population Stability

THE ability of populations to absorb perturbations is often investigated theoretically by some variant on the prey-predator, Lotka-Volterra, equations. Usually the treatment is in terms of linearised equations and thus deals with very small perturbations from a steady state. Recently, May¹ has enlarged the scope of investigation by considering perturbations from limit cycles rather than divergence from the simple steady state. This theoretical approach assumes populations uniform in space if not in time, but much of the variability found in nature is not only in large temporal cycles but in smaller-scale spatial patchiness. This variability may be handled statistically to give, for some specified area, a mean and variance for the populations. These can be used for 'sensitivity analysis' of the output from a theory or computer model, or as a basis for stochastic treatment. In either case this variability, as a function of changes in space, is not an intrinsic part of the model. Yet much experimental work shows that populations of prey or predator with limited abilities to disperse can produce spatially heterogeneous patterns, and the scale and complexity of these patterns may have some effect on persistence of these populations^{2,3}.

The technical problem in theorising about the consequences of dispersal lies in quantifying the rate of dispersal. In the sea, this can be overcome for planktonic populations such as phytoplankton and small herbivorous copepods which are almost entirely at the mercy of water movement and yet display considerable patchiness⁴. The dominant form of motion in the open sea away from areas of strong ocean currents is turbulent lateral diffusion⁵. This diffusion is similar, mathematically, to ordinary Fickian diffusion although on a much larger scale. To study the effect of this process on populations and to include the non-linear effects, it is simplest to begin with an expansion of the Lotka-Volterra equations to include variation along one spatial dimension, x . For phytoplankton, P , and herbivorous copepods, H , the interactions can be expressed as

$$\begin{aligned}\partial P/\partial t &= aP - bPH + \partial/\partial x \kappa \partial P/\partial x \\ \partial H/\partial t &= cPH - dH + \partial/\partial x \kappa \partial H/\partial x\end{aligned}$$

Assume that at some time there are random perturbations within the limits $(0, L)$ of x , expressed as Fourier series,

$$P = \sum_{n=0}^{\infty} A_n \cos \lambda_n x, \quad H = \sum_{n=0}^{\infty} B_n \cos \lambda_n x, \quad \lambda_n = 2\pi/L$$

then the time rates of change of the coefficients A_n and B_n are expressible in the form,

$$\begin{aligned}dA_n/dt &= A_n(a - \lambda^2 n^2 \kappa) - bC_n \\ dB_n/dt &= cC_n - B_n(a + \lambda^2 n^2 \kappa)\end{aligned}$$

where

$$C_n = \frac{1}{2}(\sum_{r+s=n} A_r B_s + \sum_{|r-s|=n} A_r B_s)$$

The terms involving C_n arise from the non-linear interactions of P and H . When these are ignored, as in the linearised study of small disturbances, or when only one population is considered, then perturbations at any wavelength are either amplified or damped at that wavelength and it can be shown⁶ that perturbations below a certain critical wavelength will be damped out by the processes of diffusion. In other words, only large patches would be expected to persist against the smoothing effects of turbulence.

The introduction of the non-linear terms leads to entirely different conclusions. Not only do perturbations interact to cause shorter wavelength 'harmonics' (the first term in C_n) but also they interact to give 'beat frequencies' at longer wavelengths (the second term in C_n). In particular, the perturba-

tions alter the mean. For example, for the mean value A_0 of P in $(0, L)$.

$$dA_0/dt = aA_0 - bA_0B_0 - 1/2b \sum_{r=1}^{\infty} A_r B_r$$

The first two terms on the right-hand side correspond to the normal 'Lotka-Volterra' equation⁷ which produce bounded oscillations with time. The spatial variations A_r, B_r ($r \geq 1$), however, interact to alter this mean. Thus, perturbations at any wavelength can, in theory, act to displace the prey and predator from their neutral limit cycle. In this way small-scale spatial variability in the populations which, on the linearised theory, would be damped out could, by directly displacing the mean, lead to a random walk to extinction as happens with stochastic effects on the ordinary Lotka-Volterra equations⁸ without diffusion.

As it would seem that diffusion or dispersal can never be sufficient to damp out fluctuations, these theoretical effects emphasise the need for positive stabilising factors, such as functional responses⁹ acting at the average population levels. The conclusions also imply that, when considering the effects of variability within an ecosystem, it is inadequate to introduce these by studying the effects of variation of each population on a model of the average population levels of an ecosystem. This ignores the interactions which can occur spatially and which thereby introduce changes in these average levels. Although the very simple, Lotka-Volterra, equations have been used here, more complex reactions, which introduce stabilising functions but do not consider spatial changes, should still have the same inadequacies in determining the limits of stable response in the face of environmental fluctuations.

These concepts have been developed for marine planktonic communities but the principles involved should apply to populations where dispersal is a significant feature of their life cycles. For plankton it is possible to quantify the effects on stability introduced by the combination of diffusion and spatial heterogeneity and this will be described elsewhere.

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Energy cost of gliding flight in herring gulls

By using the interplay of aerodynamic and gravitational forces, birds are known to travel considerable distances on fixed wings. The aerodynamics of this form of flight has been studied in the field¹⁻³ and in wind tunnels⁴⁻⁶, but the energetic cost is unknown. We report here experimental determinations of oxygen consumption during prolonged gliding.

Wild-trapped herring gulls (*Larus argentatus*) were trained to fly in a wind tunnel⁵ while wearing light-weight (10 g) ventilated head masks. From an initial sample of eight animals, only two would learn to glide consistently

TABLE 1 Metabolic rates during gliding flight in two herring gulls (calculated from oxygen consumption, using $1 \text{ l O}_2 \text{ h}^{-1} = 5.58 \text{ W}$)

Gull No.	Mass (kg)	No. of flights	Resting	Mean	Metabolic rate (W)		
					Gliding	Minimum	Mean increase
1	0.965	5	6.64 ± 0.58	12.50 ± 1.60		9.95	$1.9 \times$
2	0.859	4	6.57 ± 0.92	15.41 ± 0.81		14.71	$2.4 \times$

Values are means $\pm$ standard deviation.

for periods of twenty minutes without flapping. All the reported data are from these two animals. Oxygen consumption was measured by drawing room air through the mask at a metered rate of 25 l min^{-1} and measuring the difference in oxygen concentration of the gas from the mask and that in room air (for details, see ref. 8). Oxygen consumption was taken as the stable minimum value maintained for at least 5 min of the 20 min test. We assume that this stable value represents steady state oxygen consumption as no increase was apparent at the end of the gliding periods. Resting measurements were made both before and after glides on birds standing unrestrained on the floor of the wind tunnel under the same conditions of temperature and illumination. Stable minima maintained over 20 min periods were taken as resting oxygen consumption levels. Food was withheld from the birds for 16 h prior to all experiments and in all metabolic calculations, R.Q. was taken as 0.8. Air speed and glide angle were adjusted to values at which the bird had minimum acceleration relative to the tunnel. For both animals this resulted in an air speed of 10.8 m s^{-1} and a glide angle of 6.5° below horizontal. The air temperature was 27.5 to 29.2° C .

Table 1 shows results from resting and gliding birds. Oxygen consumption at rest for both birds was about 1.7 times the expected standard rate as calculated from the allometric equation of Lasiewski and Dawson⁷. For one bird, gliding resulted in a mean increase in oxygen consumption to 1.9 times the resting level and for the second bird to 2.4 times the resting level.

It is interesting to compare our measurements with those taken for steady state flapping flight under similar wind tunnel conditions. Several such determinations have given an oxygen consumption during steady state horizontal flapping flight of about seven times the resting level⁸⁻¹⁰ (this ratio will probably vary as additional species are studied). Another comparison is with Pennycuik's theoretical calculations of the cost of gliding². By adding the calculated standard metabolic rate to an estimate of the power input by the pectoral muscles during gliding, he arrived at a figure of about 1.5 times the basal rate for the white-backed vulture. This provides an interesting comparison with our minimum value of 1.5 times the resting level (compare Table 1).

We conclude that gliding flight represents an increase to about twice the resting level of oxygen consumption and has a substantial energy-saving advantage over flapping flight.

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Evidence Against a Genetical Component to Performance on IQ Tests

Eaves and Jinks¹ have failed to note the significance of Scarr-Salapatek's² data, which provides important evidence against the heritability of IQ performance. A straightforward overall evaluation of this study, together with a review of evidence³⁻⁷ which Eaves and Jinks consider to be more secure in establishing a genetical component to IQ performance shows that: (1) the upper limit of IQ heritability in Scarr-Salapatek's study is $15\% \pm 16\%$. This is consistent with zero heritability and directly contradicts the higher figures claimed by other studies³⁻⁷; (2) other studies⁵⁻⁷ which use identical-fraternal comparisons to derive apparently higher upper limits to heritability do not take into account the more similar treatment frequently given to identical twins. When this is adjusted for, these studies are consistent with low or zero heritability; (3) the similarity between separated identical twins, used by Jinks and Fulker³ to derive an 80% heritability estimate, can be quantitatively accounted for by highly correlated placement (ref. 8 and L. J. Kamin, Invited Address, Eastern Psychological Association, Washington DC, March 1973) with little or no genetical component.

We use the methods of Jinks and Fulker for our evaluation. The difference in correlation coefficients for identical twins raised together (MZ) and fraternal twins raised together (DZ) is

$$r_{MZ} - r_{DZ} = [G_1 + E_1(DZ) - E_1(MZ)] / \sigma_T^2 \quad (1)$$

where

$$\sigma_T^2(DZ) = \sigma_T^2(MZ) \text{ and } G = G_1 + G_2$$

In general $E_1(DZ) > E_1(MZ)$ since identical twins are treated more similarly than fraternal twins. Identical twins are of the same sex, are frequently dressed alike, given the same toys and mistaken for one another. Thus, large differences in correlation of identical and fraternal twins do not necessarily mean high heritability, or any heritability^{4,8}, and studies of MZ-DZ differences can only give upper bound estimates for G_1

$$G_1 / \sigma_T^2 \leq r_{MZ} - r_{DZ}$$

To obtain the upper limit heritability from Scarr-Salapatek's data we have, for the error in z scores of MZ twins, $\sigma_{MZ}^2 = a\sigma_{ss}^2 + b\sigma_{os}^2$. The coefficients a and b are given in Eaves and Jinks¹. With $\sigma_{ss}^2 = 1/(N_{ss} - 3/2)$ (ref. 9) where N_{ss} is the number of same sex pairs and similarly for σ_{os}^2 and with $\sigma_r = (1 - r^2)\sigma_z$ we compute the standard deviation of the correlation coefficient r_{MZ} . Assuming $Z_{DZ} = Z_{os}$ (this raises

the heritability estimate since in general same sex DZ twins are treated more similarly than opposite sex DZ twins) we then compute the variance, σ_d^2 , of the difference, $r_{MZ} - r_{DZ}$, $\sigma_d^2 = \sigma_{MZ}^2 + \sigma_{DZ}^2 + 2(1 - r_{MZ}^2)(1 - r_{DZ}^2)(P/(1 - 2p))\sigma_{\text{cor}}^2$ where $p = 0.30$ for whites and $p = 0.34$ for blacks. For Scarr-Salapatek's Table 8 we obtain four independent estimates of the difference $r_{MZ} - r_{DZ}$ as follows: black, lower socio-economic status (SES) = -0.165 ± 0.185 ; blacks higher SES = 0.109 ± 0.184 ; whites, lower SES = -0.046 ± 0.340 ; whites, higher SES = 0.160 ± 0.110 . Using least squares the overall best fit is

$$r_{MZ} - r_{DZ} = 0.075 \pm 0.082 \quad \chi^2 = 2.4(3 \text{ d.f.})$$

or

$$G_1 \leq 7.5\% \pm 8.2\%$$

Estimating the heritability depends on the relative magnitudes of G_1 and G_2 . Previous work has used $G_1 = kG_2$ with $k \approx 1$ (refs 1, 2, 5, 6). For purposes of comparison we choose $k = 1$ and obtain

$$h^2 \leq 15 \pm 16\%$$

which is a new upper limit to the heritable component to performance on IQ tests.

This result is to be compared with other estimates⁵⁻⁷ cited by Eaves and Jinks derived from the identical approach. It is 4.0 standard deviations lower than the figure of $h^2 = 80\%$ quoted by Jensen^{5,6} and promulgated in popular accounts¹⁰. The probability of a discrepancy this large occurring by chance is less than 10^{-4} if the true heritability is $h^2 = 80\%$.

We fail to understand why Eaves and Jinks are prepared to discard this result. To do so only perpetuates the apparently common practice in this field of ignoring or failing to report evidence against the genetic hypothesis. As Scarr-Salapatek describes it "there are few published reports of null results unless a major theoretical point is at issue. I, for one, obtained the same correlation (0.61) for blood-grouped MZ and DZ twins on an individually administered test of non-verbal IQ and did not submit the results for publication (because no one would believe that MZ twins were not more similar, there were only sixty pairs and so on)"¹¹.

The apparent discrepancy between $h^2 \leq 15 \pm 16\%$ and the higher figures reported elsewhere^{5,6} is resolved by estimating the size of the 'treatment' effect, caused by the more similar treatment of MZ twins. By comparing the correlations of groups with the same G_1 we obtain pure treatment effects of expected magnitude less than $E_1(DZ) - E_1(MZ)$. From Victorin¹² $\Delta r_{DZ}(\text{male-female}) = 0.12 \pm 0.05$ and $\Delta r_{MZ}(\text{male-female}) = 0.15 \pm 0.09$. For comparison $\Delta r(\text{males})(MZ-DZ) = 0.18 \pm 0.09$ and $\Delta r(\text{females})(MZ-DZ) = 0.15 \pm 0.05$ in the same data). Similarly from Huntley¹³ $\Delta r_{DZ}(\text{same sex-opposite sex}) = 0.21 \pm 0.09$ and from Jinks and Fulker³ $\Delta r_{MZ}(\text{boys-girls}) = 0.17 \pm 0.16$. From Ehrlenmeyer, Kimmling and Jarvik⁷ we see that the range in correlations for studies of the same groups is large. For parent-child $\Delta r = 0.6$, for siblings $\Delta r = 0.5$, and for same sex DZ twins $\Delta r = 0.45$. Since in Jensen's method⁶ $\Delta r(MZ-DZ) \sim 0.35$ yields 70% heritability, these figures demonstrate that the treatment effect acting alone is enough to produce the Δr s observed between MZ and DZ twins.

We now consider the four studies of separated twins. Kamin has demonstrated gross errors in methodology and analysis in these studies but the Shield's data is still useful for a quantitative comparison of genetic and environmental models. Jinks and Fulker³ analyse this data in terms of the components G_1 , G_2 , E_1 , E_2 , and $G = G_1 + G_2$. Naming a variance component G , however, does not mean that it is genetic. A model with the substitutions $E_T \leftrightarrow E_1$, $E_F \leftrightarrow E_2$, $E_A \leftrightarrow G_1$, $E_S(DZ) \leftrightarrow G_2$ and $E_S(MZ) \leftrightarrow G$ is an environmental model formally identical to the simple genetic model where the sources of variance are: E_T , differences in treatment of identical twins; E_A , additional differences due to the different appearance of DZ twins; E_F , between pairs family differences; E_S , differences in social environment. The results are shown in Table 1.

The large value of $E_S(MZ)$ means there is a high degree of similarity in the environments of separated MZ twins. Such

TABLE 1 Comparison of Variances of Environmental and Genetic Models Based on Analysis of Shield's Data

Environmental model		Genetic model
Variance due to differences in Social environment ($E_A(MZ)$)	71% $\pm$ 12%	Genetics (G)
Family (E_F)	0%	Family and social environment (E_2)
Within family treatment (E_T)	29% $\pm$ 12%	Within family treatment (E_1)

a conclusion is born out by Fehr's⁸ and Kamin's analysis of Shield's data. The null E_2 in the genetic model contradicts the known fact that social environments have a large effect on IQ performance¹⁴ while the null E_F in the environmental model means that differences in the way families in the same social environment treat children have little effect on IQ performance compared to differences in the way the social environment treats children. The environmental model is actually more consistent with the data than the conventional genetic model.

In summary, we note the following. The upper limit obtained from Scarr-Salapatek's data of $h^2 \leq 15\% \pm 16\%$ ($G_1 \leq 7.5 \pm 8.2\%$) is evidence against the heritability of IQ performance. Other low results go unreported¹¹ and this lends additional weight to her results. This 15% figure and every higher estimate reported in MZ-DZ studies contains an uncontrolled environmental component deriving from the more similar treatment accorded identical twins. Estimates indicate that this effect is large enough to make other upper limits derived from MZ-DZ comparisons consistent with zero. The separated twin studies when properly analysed also give heritability estimates which are low or consistent with zero.

We conclude that the evidence used to support high heritability of IQ performance actually yields low estimates of heritability consistent with zero and not larger than the upper limit of $h^2 \leq 15\% \pm 16\%$. Zero heritability of IQ performance means that the individuals in the population all have the same genetic potential for the expression of this trait.

We thank Professor Leon Kamin for making his results available prior to publication and Professors John Gibbon, Sonia Ragir and Eugene Weinstein for critical readings of the original manuscript.

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book reviews

Watch without Mother

Separation: Anxiety and Anger. Vol. 2 of Attachment and Loss. By John Bowlby. Pp. xviii+444. (The International Psychoanalytical Library No. 95.) (Hogarth and the Institute of Psycho-Analysis: London, May 1973.) £4.50.

PSYCHOANALYSIS has been long on theory and short on experimental evidence. Dr John Bowlby, the psychoanalyst best known for his work on the effects of maternal deprivation on children, has dedicated his later career to remedying that gap. He, and the institution with which he is connected, the Tavistock Institute of Human Relations, are now trying to use the techniques of scientific research to study the way in which the human personality develops. (The institute's director, Dr Dorothy Heard, was originally trained in the biosciences.) This volume is the second of a projected trilogy in which Dr Bowlby, looking at his basic theory that the loss or prolonged absence of the mother harms the child, asks why and how?

His method is to take as primary data the observation of young children in defined situations, and then to extrapolate forwards. He sees this as a reversal of the traditional direction of psychoanalytic hypothesis-making, which is to confront a fully formed personality and then to conjecture how it got that way. Nonetheless, Dr Bowlby wants it clear that his frame of reference is still psychoanalytic. The concepts which he sees as basic to the growth of personality are separation anxiety, defence, the bonds between people and so forth which, in his view, have been ignored by the behavioural sciences apart from psychoanalysis. But he is adamantly not a classical Freudian. Instead, he has a "perspective based on a Darwinian type theory of evolution." What this means in practical terms is that he is willing to use animal behaviour as evidence and in theoretical terms that he sees certain kinds of fears as functional, of survival value to the human race, rather than as infantile or neurotic.

Bowlby's exposition, however, is more interesting than his conclusions. From assembled evidence, he shows that the strong reaction to a mother's absence is shown by babies from the age of 26 weeks onward. He analyses the findings that a single separation of 6 days or more from its mother is harmful to an infant rhesus monkey, with effects observable as much as 2 years later. ("The

only safe dose", he concludes about maternal deprivation in these monkeys, "is a zero dose.") He declares unqualifiedly that while few other predictions can be made about children separated from their parents, it is clear that when a child is threatened with being abandoned (either explicitly or obliquely, by over-hearing quarrels), the effects of an actual separation when it occurs are likely to be magnified and to persist.

He also feels on sure ground when he rejects some cherished ideas—Freud's hypothesis of 1926 that the fear of mother's absence results from the infant's learning that when she is absent, his physiological needs go unmet, and the belief held by Freud and almost everybody else that an excess of parental affection can make a clinging child. The evidence is all the other way, he says, with documentation, that overdependence is the result of bitter experience, not excess of gratification. So why does the unwilling separation from its mother or her substitute make a child respond with fear? Bowlby offers tentative conclusions and calls for more research. His hypotheses are (1) that such fear may be instinctive, (2) the infant may learn to associate its mother's absence with distress just as it learns to associate her presence with comfort and (3) an infant may learn that it is more afraid of fear-arousing situations, such as a loud noise, when its mother is absent and thus associate her absence with fear.

Bowlby once again offers a more kindly, less punitive approach to the child than is generally taken, either by the post-Victorians or the Freudians. He deplores the fact that most people still believe that a normal child should not make a fuss when its mother leaves it at school and that "babyishness" is something to be corrected. Or that something as positive as parental love for a child could cause the pathology of overdependence.

Dr Bowlby's words are so influential—he kept a whole generation of mothers home from work—that they have to be weighed not only in the light of their contribution to psychoanalytical theory but of what will be made of them by the public. The law, the schools and many parents still have scarcely a glimmer of perception that anxiety in children cannot be cured by preaching self-control. Dr Bowlby's humane—and rational—explanations of the origins of the child's anxiety need to be listened to. But at the same time he does raise

spectres of smothering, of the claustrophobic nuclear family of which Dr Edmund Leach warned in Reith Lectures a few years ago. Is a zero dose of maternal deprivation a good thing?

BRENDA MADDOX

Ethereal molecules

Molecules in the galactic environment. Edited by M. A. Gordon and L. E. Snyder. Pp. xv + 475. (Wiley: New York and London, October 1973.) £9.50.

"ALL of the elements that make up our bodies (with the exception perhaps of hydrogen) have been formed in stars. Thus each and every one of us is, in a very real and literal sense, a little bit of stardust." These words of Willy Fowler give the clue to the wide current interest in interstellar molecules for it is believed that they may be an important link in the evolution of life in the Universe. Of course even the largest molecules— $\text{CH}_3\text{C}_2\text{H}$ (methylacetylene); HCOCH_3 (acetaldehyde)—found by radio searches in the interstellar medium are a long way from the truly complex molecules which characterise living organisms. Nevertheless, since molecule synthesis must begin somewhere, it is gratifying that the first steps of this synthesis are amenable to investigation in the depths of interstellar space even if later steps are forever lost in the uncharted atmosphere or sea of some early planet. But who really knows? Ten years ago nobody would have predicted that such a rich field of interstellar chemistry lay around the corner—a veritable Black Cloud just waiting to be discovered.

In November 1971 a conference to discuss recent discoveries was held at the National Radio Astronomy Observatory in Charlottesville, USA. This book is the conference report edited by two astronomers who have been in the forefront of the radio discoveries.

In addition to the biological implications another important aspect of recent work is the interstellar chemistry of molecule formation (and destruction). Available observations give crucial information about densities, temperatures and excitation conditions in the molecule-bearing clouds. These allow some appraisal of the proposed theories of molecule formation. It is clear that a variety of processes are at work—some are surface reactions on dust grains and others are gas-phase reactions of varying complexity, but we are still far from a complete understanding of all the proc-

esses at work in interstellar chemistry. There is no indication of how complex the undiscovered interstellar molecules may be. Even so it is unlikely that DNA will ever be discovered in interstellar space.

One field of study scarcely touched upon in this volume is the use of molecular observations as probes of the interstellar medium. They have proved particularly significant in studies of dense gas clouds such as those found in the general interstellar medium, in the immediate vicinity of certain recent formed stars and in the galactic centre region.

In summary, this book provides a number of excellent review articles—especially those of Field, Iben, Litvak and Ponnampertuma, and a medley of research reports. It will be read with interest by astronomers and biologists.

R. D. DAVIES

Strong probability

Stochastic Analysis: A Tribute to the Memory of Rollo Davidson. Edited by D. G. Kendall and E. F. Harding. (Wiley Series in Probability and Mathematical Statistics.) Pp. xiii + 463. (Wiley: London and New York, August 1973.) £11.

NAMES of branches of science are often coined to express an insight into their nature, and 'stochastic analysis' is one of these. It witnesses to the end of the isolation of probability theory, to its penetration into, and its complementary illumination by, many different areas of pure and applied mathematics. Today one finds probabilistic methods cropping up in the most surprising places, just as probability theory itself now calls on mathematical tools of every variety.

Twelve years ago the University of Cambridge established a chair of mathematical statistics, and shortly afterwards moved its Statistical Laboratory from the basement of the chemical laboratories to a disused paper warehouse. In such unpromising surroundings there grew up the leading centre in Britain for research in stochastic analysis, attracting visitors from all over the world. This volume, which is to be joined by a companion on stochastic geometry, gives a good feeling for the exciting work that has gone on there. It originated in the idea of a memorial to Rollo Davidson, a brilliant mathematician whose tragic death in a mountaineering accident at the age of twenty-five deprived the subject of one of its most promising workers, and his friends of a delightful companion. Among the articles printed is posthumous work of Davidson, reconstructed from the papers which he left.

Although in one sense the book is historical, it is not backward-looking; the lines of research described are still very active, and there are many unsolved problems which pose a challenge

for the future. Nor, despite the fact that a dozen authors have contributed, does the book exhibit the miscellaneous character common in such collections; the influence of the warehouse, and of its director, runs as a unifying thread through the various papers. To Professor Kendall, and to his colleague Dr Harding, one owes a debt of gratitude for producing a book which will be of great interest not only to the specialist, but to the mathematician or statistician who wants to know what is going on in this field.

J. F. C. KINGMAN

Atmosphere and climate

Synoptic Climatology. By R. G. Barry and A. H. Perry. Pp. xvi + 555. (Methuen: London, October 1973.) £9.50.

MANY readers will have only a hazy idea of what is meant by synoptic climatology; indeed different authors have different ideas on the subject but in this volume the basic aim is to relate local or regional climates to a meaningful frame of reference—the atmospheric circulation. The authors recognise two stages in any synoptic climatological study, the determination of categories of atmospheric circulation type and the assessment of weather elements in relation to these types. Such studies cover a very broad field including long range weather forecasting on time scales from a week or so ahead to a decade or more.

The book is sensibly laid out with main sections covering history, data and analysis, synoptic climatological analysis, statistics, applications and future prospects. One of the best features is the bibliography of some eighty pages which covers much of the useful foreign literature as well as most of the modern work of the British Meteorological Office and includes works published up to 1973. I could detect no errors in the bibliography.

The volume is intended for students of advanced climatology at senior undergraduate or graduate level and for students of environmental problems. The authors have not, however, differentiated between the wheat and the chaff; they refer to important and unimportant aspects of the subject without a clear indication of which are the most essential. In chapter 2, for example, there is a good deal of obsolescent information dating from before 1950 about the frequency of cyclones and anticyclones and the tracks of depressions and only a few pages are devoted to discussion of the value of satellite pictures in synoptic climatological analysis.

Chapter 3 gives on the other hand a very good account of the various classification systems which have been devised to describe day to day weather on both regional and hemispheric scales.

Not only are the more well known classifications such as those of Lamb and the German Grosswetterlagen fully discussed but the various Russian methods are explained in a lucid manner which is not easy to do from translations of the originals which I have studied. Lesser known classification systems, such as those based on both surface and upper air conditions, for Italy by Gazzalo and for Switzerland by Schuepp, are welcome inclusions.

The chapter on statistical methods is rather patchy; the more elementary side is adequately covered but the chapter tries to cover too much ground which would be better dealt with in a suitable text book.

Since synoptic climatology is essentially a practical subject the devotion of 150 pages to applications is very reasonable; in fact this chapter could easily have been made longer. The best use has not, however, been made of the available space: there is far too much discussion about "singularities" and methods of defining natural seasons (curiously without reference to the Russian six-season system) and very little on important applications such as animal diseases, (only one reference to the extensive work of L. P. Smith) and human biometeorology.

The section on long-range forecasting gives some indication of the complex methods presently used, but does not give any clear idea of the relative importance of the different ideas. There is a fairly good section outlining the possibilities of climatic forecasting on the time scale of decades or longer and there is good appreciation of the importance of sea temperature anomalies in determining the various atmospheric modes.

The final chapter, though short, is an adequate summing up and indicates that there is a promising future for the subject in many fields. The book should certainly go a long way towards meeting university teaching needs and will be read with interest by many meteorologists. It is a pity that some of the figures are not clear, due usually to coastlines not being adequately distinguished from other lines but occasionally also to inadequate explanation; there are also a few typescript errors. Nevertheless the book is a welcome addition to the scientific literature.

R. A. S. RATCLIFFE

Erratum

In the review of the book *Immunopotentialization* (Ciba Foundation Symposium 18) (242, 322; 1974), the third paragraph should have started: "It seems likely that the agents useful in cancer act on the immune system" instead of "It seems unlikely . . ." as printed.

matters arising

Haem deficiency and chain synthesis

SIR—The results reported by Giglioni *et al.*¹ on the differential effect of haemin on α and β chain synthesis are interesting. The authors conclude that only α -chain synthesis is stimulated by haemin. This contrasts with the conclusions of others^{2,3} that in a cell-free

that the selective effect of haemin acted at the level of translation, supports our earlier hypothesis explaining the relative depression of α chains. In haem-free cell-free systems, globin synthesis is inhibited after several minutes. This has been shown to be due to a soluble factor which inhibits the initiation of globin chains⁸⁻¹⁰. As α chains are normally initiated at a

⁹ Gross, M., and Rabinovitz, M., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 1565 (1972).

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TABLE 1 Relative rates of α and β chain synthesis by reticulocytes of patients suffering from haem deficiency disorders

Condition	No.	Mean $\alpha:\beta$	Effect of exogenous haemin
Congenital sideroblastic anaemia	3	0.5–0.8	0.85–0.90
Acquired sideroblastic anaemia	6	0.7–0.81	0.90–0.98
Lead poisoning	4	0.4–0.7	0.8–0.91*
Lead <i>in vitro</i> (1.5 $\mu\text{g ml}^{-1}$)	15	0.75–0.8	0.95–1.05
Iron deficiency	6	0.82–0.94	—

The α and β chains were isolated from globin prepared from unpurified whole cell lysates after a 60 min incubation with ³H-leucine.

* Observations on the reticulocytes of two patients.

system the stimulatory effect of haemin on protein synthesis is non-specific but is consistent with work, previously reported from this laboratory⁴⁻⁶, on the relative rates of synthesis of α and β chains in reticulocytes of patients who were haem deficient.

In 1971, reticulocytes of patients with congenital and acquired sideroblastic anaemia were found to show a low $\alpha:\beta$ ratio⁴. Later a similar depression in $\alpha:\beta$ ratio was found in children with acute lead poisoning⁵; an effect which could be produced by incubating normal reticulocytes in the presence of lead⁶. In all three situations the $\alpha:\beta$ ratio reverted to unity on addition of exogenous haemin. The results from all these four studies are summarised in Table I. Although these results contrast with observations of Tavill *et al.*⁷ using rabbit reticulocytes, we conclude that in human reticulocytes which are haem deficient, synthesis of α chains is depressed more than β chain synthesis. Thus, haem must have a differential effect upon the synthesis of these two chains⁴.

The conclusion of Giglioni *et al.*¹

slower rate than β chains¹¹, their initiation might be more sensitive to the action of the inhibitor.

Yours faithfully,

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³ Mathews, M. B., Hunt, T., and Brayley, A., *Nature new Biol.*, **243**, 230 (1973).

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⁶ White, J. M., and Piddington, S. K., *Clin. Sci.*, **44**, 20 (1973).

⁷ Tavill, A. G., Grayzel, A. I., London, I. M., Williams, M. K., and Vanderhoff, G. A., *J. biol. Chem.*, **243**, 4987 (1968).

⁸ Maxwell, C. R., and Rabinovitz, M., *Biochem. biophys. Res. Commun.*, **35**, 79 (1969).

Controlled use of nucleolus organisers

SIR—Macgregor has proposed that only some of the nucleolus organisers present act as the templates for transcription of rRNA in somatic cells, for the amplification of ribosomal genes (rDNA) in oocytes, and for repeated replication of rDNA in polytene chromosomes¹. This hypothesis evades the usually accepted requirement for a compensatory increased use of one organiser if the other one is defective or missing. There must, however, be regulatory devices to limit such usage, for example, a quantitative control of transcription, which do involve compensatory changes, and so the hypothesis provides no genuine economy of assumptions.

As in many other eukaryotes, a large proportion of diploid cells in *Xenopus* tadpoles have two nucleoli, both of which are engaged in RNA synthesis simultaneously^{2,3}. This is the evidence, overlooked by Macgregor, that both organisers are normally transcribed. As tadpoles which have only one organiser and form one nucleolus in each cell still achieve a normal rate of rRNA synthesis, it is hard to avoid the conclusion that the rate of transcription of the solitary organiser has doubled. Even fragments of organisers are active in heterozygotes, where they form miniature nucleoli⁴. Macgregor's hypothesis concerning transcription has thus a very limited application, being identical to the concept of latent organisers and known only in some interspecific hybrids. This allelic repression of one organiser has long been suspected to indicate a control system in plant hybrids⁵, but its molecular aspects are better demonstrated in mammalian cell hybrids⁶ and *Xenopus* hybrids⁷. These examples all provide evidence of compensatory changes occurring under a transcriptional control which does not discriminate between identical organisers nor apparently reduce their activity much below the normal rate,

as the presence of extra organisers in polyploid and aneuploid plants results in extra nucleoli and a proportionate increase in RNA^{8,9}.

Many of these examples can be repeated in terms of amplification, so it is only reasonable to suppose amplification is regulated in a similar manner to that outlined above for transcription. Macgregor¹ argues to the contrary, but lacks evidence; his observations on polyploid oocytes are ambiguous on this point and I doubt if his results on spermatocytes are even pertinent.

Similarly, when applied to polytene chromosomes, Macgregor's hypothesis is only a possible but unnecessary complication to the control mechanism envisaged by Spear and Gall¹⁰.

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¹ Macgregor, H. C., *Nature*, **246**, 81-82 (1973).

² Wallace, H., *J. Morphol.*, **112**, 261-278 (1963).

³ Wallace, H., *Nat. Cancer Monogr.*, **23**, 425-430 (1966).

⁴ Miller, L., and Gurdon, J. B., *Nature*, **227**, 1108-1110 (1970).

⁵ Wallace, H., and Langridge, W. H. R., *Heredity*, **27**, 1-13 (1971).

⁶ Bramwell, M. E., and Handmaker, S. D., *Biochim. biophys. Acta*, **232**, 580-583 (1971).

⁷ Honjo, T., and Reeder, R. H., *J. molec. Biol.*, **80**, 217-228 (1973).

⁸ Lin, M., *Chromosoma (Berl.)*, **7**, 340-370 (1955).

⁹ Longwell, A. R. C., and Svihla, G., *Expl. Cell Res.*, **20**, 294-312 (1960).

¹⁰ Spear, B. B., and Gall, J. G., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1359-1363 (1973).

obituary

V. C. A. Ferraro

V. C. A. FERRARO, who died on January 3, 1974, had been Professor of Applied Mathematics at Queen Mary College, University of London, since 1952; he expected to retire from his chair later this year. He had earlier (1947-52) been professor at the University of Exeter.

When he came to Queen Mary College the Department of Mathematics had six members; there are now three separate departments of Pure Mathematics, Applied Mathematics, Computer Science and Statistics, with a total of thirty-five lecturing staff. Professor Ferraro directed this expansion with energy and foresight, encouraging the growth of algebra, magnetohydrodynamics and astrophysics, the theory of computation and statistics.

He was best known for his share in the Chapman-Ferraro theory of magnetic storms, and for his discovery of the law of isorotation in cosmic masses. The Chapman-Ferraro theory was first advanced in 1930, when Ferraro was a

research student, and he returned to develop aspects of it over many years. The theory ascribed magnetic storms to streams emitted from the Sun into otherwise empty space, and the recognition that the solar wind flows all the time has meant that considerable modifications have later proved necessary. Moreover, the theory was a theory of the initial phase of a storm only; the type of ring current required to explain the main phase was not identified until much later. The essentially valuable new idea introduced in the theory was in its recognition that a solar stream must be regarded not as a collection of individual particles, but more nearly as a perfectly conducting fluid, able to modify the geomagnetic field by pushing the field-lines in front of it. The theory also introduced the idea of a magnetospheric cavity, though a temporary one.

The law of isorotation (1937), later much used in the theory of star formation, asserts that in a cosmic rotating mass of ionised gas penetrated by a magnetic field the angular velocity rapidly tends to become constant along

a magnetic field line. This result, like those of the theory of magnetic storms, seems today an almost obvious illustration of the principles of magnetohydrodynamics (MHD). But these principles were not understood when Ferraro made his discovery; he can fairly be claimed to be one of the pioneers of MHD.

In the postwar years Ferraro did much to encourage research in MHD, by his own example, by organising regular seminars and by the writing of a book on the subject with Plumpton (1961). He also is known for his book on Electromagnetic Theory (1953). He was a person of continuous activity; this activity was a little circumscribed after a heart attack in 1965, but he was publishing papers on the solar wind and related subjects up to the end.

But he will be remembered most in the College for his old world charm and personal courtesy, which is best conveyed in the words of one of Queen Mary College's cockney waitresses "He was a real gentleman, he was".

He leaves a widow and one son.

Announcements

Awards

The Trustees of the **Lady Tata Memorial Trust** invite applications for **Fellowships** and **Scholarships** for research on leukaemia, in the Academic Year beginning October 1, 1974.

The prizewinners of the **Feldberg Foundation** awards for 1974 are **Professor G. R. Brindley FRS**, Honorary Director of the MRS Neurological Prostheses Unit, at the Institute of Psychiatry,

and **Professor P. Karlson**, Physiologisch-Chemisches Institut der Universität, Lahnberge.

Errata

In the article 'Effect of Muscle Stretching on Tension Development and Mechanical Threshold during Contractures' by H. Gonzalez-Serratos, R. Valle and A. Cillero (*Nature new Biol.*, **246**, 221; 1973) paragraph 10, line 2 should read 'depolarisations produced with solutions containing 80 to 190 nM K⁺...'. Reference 2 should read volume 117 not 177.

In the article 'The Fluorescence of Quinacrine Mustard with Nucleic Acids' by Ritva-Kajsa Selander and Albert de la Chapelle (*Nature new Biol.*, **245**, 240; 1973) the labelling to the ordinate of Fig. 4 should read 'Fluorescence units at 514 nm' not 'Fluorescence at 514 nm (F)'. In Table 1 the heading of columns 2 and 3 should read 'Fluorescence units at 514 nm' not 'Fluorescence (F) at 514 nm'. The last sentence in the legend to Table 1 beginning 'Fluorescence was expressed...' should be deleted. The name of the first author should be Ritva-Kaisa Selander.

International Meetings

March 7, **Wild Oats, Economics, Biology and Herbicides** (D. T. Sagers, Fisons Agrochemical Division, Chesterford Park Research Station, Great Chesterford, Essex) (Venue changed from Shell Centre to British Museum (Natural History))

March 11-14, **Fast Reactor Power Stations** (Mr P. McLaren, Technical Secretary, c/o Fast Reactor Training Centre, Dere, Thurso, Caithness, Scotland)

March 22-25, **Practical Metallic Composites** (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20 9LW)

March 23-April 1, **East African Community Symposium on Aquatic Resources and Central Africa** (Dr J. Okedi, Director E.A.F.S.R.O., P.O. Box 343 JINJA, Uganda)

March 25-27, **Aslib Coordinate Indexing Group Conference** (Miss Verina Horsnell, Polytechnic of North London, 207-225 Essex Road, London N1 3PN)

March 25-27, **Moving Boundary Problems in Heat Flow and Diffusion** (The Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY)

March 25-29, **Petrophysics: The Physics and Chemistry of Minerals and Rocks** (W. F. Mavor, Administrative Assistant, School of Physics, The University, Newcastle upon Tyne, NE1 7RU)

March 26-28, **Nucleation, Folding and Interactions of Biopolymers** (Dr R. H. Pain, Department of Biochemistry, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne NE1 7RU)

March 26-27, **The Conservation of Materials** (Mr C. J. A. Preuveneers, Conference Secretary, Education Centre, B. 455 AERE Harwell, Didcot Berkshire OX11 0QJ)

March 26-28, **6th Cranfield Fluidics Conference** (Organising Secretary, 6th C.F.C., BHRA Fluid Engineering, Cranfield, Bedford MK43 0AJ)

March 27, **Corrosion in the Food Industry** (Mr K. Farrow, Fulmar Research Institute, Stoke Poges, Buckinghamshire) (Altered Date)

March 27-28, **International Technico-Economical Symposium** (i.b./c.c. Administration, Nieuwelaan 65, B-1820 Strombeek, Belgium)

March 27-28, **Rubber and Elasticity Symposium** (The Registrar, University of Manchester Institute of Science and Technology, P.O. Box 88 Manchester, M60 1QD)

March 27-28, **Speckle Phenomena and their Applications** (Meetings Officer, The Institute of Physics, 47, Belgrave Square, London SW1X 8QX)

March 27-29, **Nuclear Structure and High Energy Physics** (Dr P. J. Negus, Department of Natural Philosophy, The University, Glasgow G12 8QQ)

March 27-29, **Minicomputer Interfacing** (Dr Yakup Paker, Polytechnic of Central London, 115 New Cavendish Street, London W1M 8JS)

March 28-29, **Biogenesis of Chloroplasts and Mitochondria** (Dr B. M. Richards, Searle Research Laboratories, Lane End Road, High Wycombe, Buckinghamshire)

March 28-29, **Recent Developments in the Petroleum Industry** (C. H. Maynard, Assistant General Secretary, Zoological Society of London, Regents Park, London N.W.1)

March 29, **The Genetical Society Brighton Meeting** (M. A. Ferguson-Smith, Institute of Genetics, University of Glasgow, Church Street, Glasgow G11 5JS)

March 31-April 5, **Electronics in Medicine** (Centre for Extension Studies,

University of Technology, Loughborough, Leicester LE11 3TU)

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Freshwater Biological Association. Scientific Publication No. 28: **A Key to the Adults of the British Trichoptera**. By Dr T. T. Macan. Illustrated by C. Joan Worthington. Pp. 151+5 plates. (Amble-side, Westmorland: Freshwater Biological Association, The Ferry House, Far Sawrey, 1973.) £1.25. [611]

Ministry of Agriculture, Fisheries and Food. Pest Infestation Control: Combined Report of the Director of the Pest Infestation Control Laboratory, 1968-70. Pp. viii+205+12 plates. (London: HMSO, 1973.) £1.30 net. [811]

Bulletin of the British Museum (Natural History). Entomology. Vol. 28, No. 6: **The Higher Classification of the Lycaenidae (Lepidoptera)—a Tentative Arrangement**. By J. N. Eliot. Pp. 371-505+6 plates. £6.90. Zoology. Vol. 25, No. 8: **Relationships of the Palearctic Lizards Assigned to the Genera *Lacerta*, *Algyroides* and *Psammadromus* (Reptilia: Lacertidae)**. By E. N. Arnold. Pp. 289-366. £3.35. (London: British Museum (Natural History), 1973.) [1341]

1974 Calendar—British Birds by British Artists. (London: British Museum (Natural History), 1973.) 75p. [1311]

Other Countries

American Geographical Society. Antarctic Map Folio Series. Folio 16: **Morphology of the Earth in the Antarctic and Subantarctic**. By Bruce C. Heezen, Marie Tharp and Charles R. Bentley. Pp. 16+8 plates. Folio 17: **Marine Sediments of the Southern Oceans**. By H. G. Goodell, R. Houtz, M. Ewing, D. Hayes, B. Naini, R. I. Echols, J. P. Kennett and J. G. Donahue. Pp. 18+9 plates. \$11. (New York: American Geographical Society, 1972 and 1973.) [2910]

Conservation for Survival: Ethiopia's Choice. By Leslie H. Brown. Pp. ii+96. (Addis Ababa: Haile Sellassie I University, 1973.) £1.20; \$3. [3010]

Fisheries Research Board of Canada. Technical Report No. 411: **Variation in the Food of American Plaice (*Hippoglossoides platessoides*) with Fish Length and Locality in the Scotian Shelf and Gulf of St. Lawrence**. By J. S. Scott. Pp. 15. (St. Andrews, NB: Fisheries Research Board of Canada, Biological Station, 1973.) [3010]

Australian Journal of Zoology. Supplementary Series No. 21: **Studies on Australian Muscoidae (Diptera). IV: A Revision of the Sub-Families Musconae and Stomoxysinae**. By A. C. Pont. Pp. 129-296. (East Melbourne: Editorial and Publications Section, CSIRO, 372 Albert Street, 1973.) [3010]

United States Department of the Interior: Geological Survey. Professional Paper 529-K: **Atlantic Continental Shelf and Slope of the United States—Sand-Size Fraction of Bottom Sediments, New Jersey to Nova Scotia**. By James V. A. Trumbull. Pp. iii+45. (Washington, DC: Government Printing Office, 1972.) 55 cents. [3010]

TABLE 1 Observations of λ -Scn

Date	Observation starting time (UT)			No. of integration periods	Source count	Background count
	h	min	s			
19/9/72	21	8	35	116	329	325
20/9/72	9	58	30	164	557	574
20/9/72	21	19	3	129	396	412
21/9/72	9	11	18	152	478	493
21/9/72	21	16	32	140	397	407
27/4/73	16	6	34	100	273	323
30/4/73	18	52	3	63	185	213
1/4/73	8	3	36	172	598	574
1/4/73	19	40	0	59	217	193
2/4/73	7	17	50	140	478	461
Totals	109 h			1,235	3,908	3,975

One integration period is 62.7 s long and the time between the start of successive integration periods is 86.5 s.

Table 1 of the article "Upper limit to the flux of soft X rays from λ -Scn" by K. T. Strong, M. W. Colley and J. L. Culhane which appears on page 34 of this issue. Due to production difficulties this table was unfortunately omitted from page 34.